Studies of zinc nutrition in lambs with special reference to plasma bound zinc.

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STUDIES OF ZINC NUTRITION IN LAMBS WITH SPECIAL REFERENCE TO PLASMA BOUND ZINC

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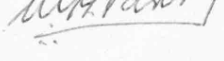

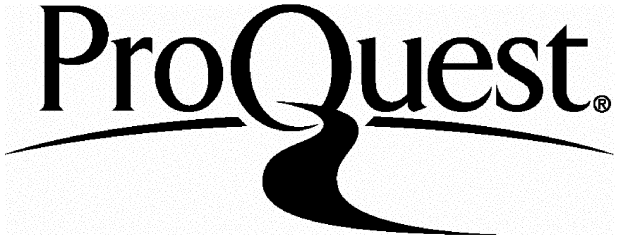

CONTENTS

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SUMMARY

1. The effect of feeding a severe Zinc deficient diet (0.9 mg Zn./Kg) to lambs was investigated by assessing performance, plasma proteins, plasma bound and unbound Zinc.

2. Performance decreased, and results indicated an inability to adapt to the deficient conditions in six weeks. Food conversion was three to four times less efficient than in the control lambs, growth rate was significantly lower after three weeks, and food intake decreased in the first week. Rapid recovery of performance occurred when Zinc supplemented diets (40 mg Zn./Kg) were fed.

3. External symptoms of Zinc deficiency showed some relation to plasma alkaline phosphatase activity which decreased during six weeks. Pin-prick sized haemorrhages were observed in the same week that enzyme activity decreased significantly. An explanation of the haemorrhages was suggested by reference to published work by the author.

4. Total plasma protein concentration increased significantly each week. This was mostly due to a rise in globulin concentration, whilst albumin concentration was decreased. Both these proteins showed normal values after five weeks of feeding Zinc supplemented diets. This suggested that Zinc stimulated the synthesis of albumin, and inhibited the synthesis of globulins.

5. Evidence was obtained to suggest that Zinc deficient female lambs had a more efficient mechanism than male lambs, of homeostatic regulation of plasma Zinc, α1- and α2-globulins.

6. Evidence showed an interrelationship between total plasma Zinc, plasma protein bound and unbound Zinc.

7. Protein bound Zinc in normal lamb plasma was located in albumin and α2-macroglobulin.

8. Evidence was obtained which suggested a possible homeostatic mechanism controlling bound and unbound plasma Zinc in Zinc deficient lambs. The amount of albumin bound Zinc decreased rapidly, whilst the α2-macroglobulin bound Zinc was retained for a longer time. The amount of unbound Zinc remained relatively constant throughout. Therefore, the evidence suggested that albumin was releasing Zinc to maintain the proportion of Zinc necessary for physiological functions.

CHAPTER 1

Introduction

It is generally accepted in veterinary sciences that the certainty and speed with which a disorder in farm animals, arising from deficiency or excess of a dietary component, can be recognised depends greatly upon the clarity with which the clinical consequences of this disorder have been characterized in experimental studies. In most of the numerous disorders attributable to deficiency of trace elements the signs are insufficiently specific to achieve a satisfactory diagnosis by consideration of a single gross lesion. Thus progress towards identification of the disorder must be achieved by more detailed consideration of the variety of clinical and metabolic lesions.

There have been inadequate experimental studies of lambs with Zinc deficiency. Work by Underwood and Somers (1969), with several species has shown the sensitivity of the young growing animal to a suboptimal Zinc supply, has illustrated that the demands to meet specific metabolic functions may differ greatly. They found, for example, that spermatogenesis was depressed by even mild Zinc deficiency, in the absence of other detectable lesions. The consequence of this could be poor reproductive performance in farm livestock which would support a suggestion made by Davies and Baker (1974) after extensive survey work that deficiency of some trace elements may be more widespread than hitherto believed.

It has also been emphasized by Mills (1974) that failure to achieve an adequate description of the relationship between changes in trace element supply and the response of the animal, is responsible for many of the difficulties in assessing the impact of the trace elements upon animal health. This is supported in FAO/WHO (1974) Animal Health Year Book, in that of 157 countries reporting on the incidence of animal diseases only thirty provided evidence suggesting adequate appraisal of the significance of trace element disorders.

A complete review and citation of the literature pertinent to the role of Zinc nutrition and metabolism in animals would be beyond the scope of this presentation. The objective in this thesis, therefore, has been to present a limited review of dietary Zinc deficiency mainly in ruminants, and its subsequent effects in experiments on performance and blood plasma constituents. Several reviews are available which cover a broader approach to the physiology and biochemistry of Zinc in various species. These include work by Prasad (1966) on Zinc Metabolism, by Vallee (1969) on Biochemistry, physiology and pathology of Zinc, by Mills et al. (1969) on Metabolic role of Zinc, and by Miller (1970) on Zinc Nutrition of cattle. Data for conducting nutritional and metabolic experiments with Zinc deficient animals has been described by Mills (1970). Many of these recommendations were used by the author in designing his experiments in 1971, when his research commenced. These recommendations included the adoption of a paired-feeding regime for control animals because of the

poor food consumption which was closely associated with the development of Zinc deficiency.

The following review of literature is confined to Zinc deficiency occurring naturally, and by induction on semi-synthetic diets; the effects of Zinc deficiency on performance, plasma proteins, and plasma bound Zinc; the location and distribution of plasma bound Zinc in normal metabolism and finally a consideration of Zinc homeostasis.

CHAPTER 2

Review of Literature

The first report to mention the importance of Zinc in nature was made by Raulin in 1869. He described Zinc as an indispensable nutrient for the growth of the mold Aspergillus niger. This occurred 65 years before Todd, Elvehjem and Hart (1934) published evidence that Zinc was essential for rats. In the same year Bertrand and Bhattacherjee (1934) showed Zinc to be essential for mice. For more than 20 years after these results were published Zinc was not a subject of very active nutritional investigation, probably because no Zinc deficiency syndrome was apparent in animals. This is evident from textbooks on animal physiology and animal nutrition by Dukes (1955) and Maynard and Loosli (1956) respectively which stated: "Zinc is an essential element but Zinc deficiency is not a practical problem."

At this time, however, major losses of pigs due to parakeratosis had been occurring for many years; it was recognised eventually as being due to Zinc deficiency by Tucker and Salmon (1955). Later, Zinc deficiency was shown in poultry by O'Dell, Newberne and Savage (1958) and was induced experimentally in both calves and lambs by Mills, Dalgarno, Williams and Quarterman (1967).

There are two complementary methods of investigating the nutritional role of an element in animals. Firstly, cases can be sought where the element is deficient in the animal's diet under naturally occurring conditions;

secondly, semi-synthetic diets can be fed whereby the level of the element consumed by the animal can be varied. Clearly, the second method is more easily quantitative and suitable for controlled conditions. The first method remains, however, an essential complement to allow comparison of symptoms in animals made deficient by the semi-synthetic diet.

The first report of a naturally occurring deficiency of Zinc in ruminants was made by Legg and Sears (1960). The deficiency was seen in cattle grazing forage on sandy soils in British Guiana particularly where the predominant grass was Pangola grass (Trachypogon polymorphus). Other reports of naturally occurring deficiencies include those of Haaranen (1963) in Finland and Grashius (1964) in the Netherlands; Pierson (1966) with sheep in the U.S.A. and Semenkhanov (1972).

Research reports dealing with production of Zinc deficiency in sheep on semi-synthetic diets include those of Ott et al. (1964; 1965); Mills et al. (1967); Arora et al. (1968 a,b.); Underwood and Somers (1969). Work with goats include those by Miller et al. (1964; 1966 a,b,c.; 1968b); and on cattle by Miller and Miller (1960; 1962); Miller et al. (1965 b,c; 1966 a,b; 1967a,; 1968 a,b.); Mills et al. (1967) and Pitts et al. (1966).

The external symptoms of Zinc deficiency are similar in both the naturally occurring deficiency and in the syndrome developed by feeding Zinc deficient semi-synthetic diets. From these reports, however, some differences exist, between different ruminants and also

there is a difference which depends on the severity of the deficiency.

In naturally occurring Zinc deficiencies, itching has been described by Haaranen (1963) as an early symptom of Zinc deficiency in cows. Later, Hyppola (1966) reported that itching cows had a greater incidence of difficult conceptions and abnormal oestrus. He attributed these symptoms to Zinc deficiency. He also observed that there was a tendency for cystic degeneration of the ovary, and that the retention of placenta after calving was more prevalent than in non-itching cows.

In naturally occurring Zinc deficiency, Legg and Sears (1960) observed a parakeratosis which rapidly spread over forty per cent of the body surface of cattle especially the muzzle, vulva, anus, top of tail, ears, back of hindlegs, flanks, knee and neck. Their rate of growth was also decreased.

Naturally occurring Zinc deficient sheep, observed by Pierson (1966), showed most of the previously mentioned symptoms and included loss of wool and the development of thick, wrinkled, pink skin.

During experimental studies on calves fed low Zinc semi-synthetic diets, Miller et al. (1970) and Mills et al. (1967) observed that excessive salivation and proliferation of bacteria on the surface of the tongue was one of the earliest external symptoms of Zinc deficiency. Other symptoms noticed early in the deficiency, in addition to the above were, rough hair, swelling of the feet and legs, loss of hair on the rear legs and lesions in the skin

around the hooves. Later in the deficient state, a stiff gait, swelling of the hocks and knees, wrinkled appearance or the skin as well as red, scabby appearnace to the skin has been reported. Some calves were reported as having a vision impairment. Inflammation of the skin around the nose and mouth with submucosal haemorrhages were also observed.

Similar symptoms were observed by Mills et al. (1967) in lambs fed low Zinc semi-synthetic diets, although they did not observe any skin inflammation or submucosal haemorrhages.

These external clinical symptoms were associated with the Zinc concentration of the diet and have been used by some workers in attempts to assess an optimum level of dietary Zinc which would alleviate the symptoms of Zinc deficiency without giving excess Zinc, whilst maintaining the animal in health and production. No general agreement has been reached on a suitable level of dietary Zinc for these purposes in one species. Experimental evidence shows a considerable variation in dietary Zinc requirements. Miller et al. (1962) showed that 25 mg Zn./Kg. diet was sufficient for six month old calves, whilst Miller et al. (1963) reported that one year old bulls did not require more than 8.7 mg Zn./Kg. dietary Zinc. Work by Ott et al. (1964; and 1965) showed that 2.7 mg Zn./Kg. dietary Zinc was inadequate for either calves or lambs because visible symptoms of Zinc deficiency became apparent. These were mainly loosening of the hair in calves and the wool in lambs, followed by swelling of the area between the hocks

and the hoof, lesions of the skin above the hoof and around the eyes. These symptoms agreed with those describing parakeratosis in pigs by Kernkamp and Ferrin (1953).

Experimental results by Ott et al. (1964; and 1965) also showed that the time in which the external symptoms became apparent were not consistent between the calves and lambs, despite the fact that the diets were similar in Zinc content. This would suggest that the dietary Zinc was not necessarily an indication of the physiologically available Zinc.

Whilst Ott et al. (1965) suggested that 103 mg Zn./Kg. diet was sufficient to alleviate external symptoms of Zinc deficiency in calves and lambs, Mills et al. (1967), however, suggested that only 10 to 14 mg Zn./Kg. diet was necessary. The diets used by these different workers were similar in respect to all the constituents, with the exception of the Zinc content. Therefore it must be assumed that all the dietary Zinc was available for absorption by the animals.

Although the external symptoms of Zinc deficiency are useful for assessing the general condition of the animal, the criteria of weight gain and food intake have been more important in assessing the performance of the animal.

In the experiments by Ott et al. (1965), their lambs showed a greatly reduced weight gain with 2.7 mg Zn./Kg. diet as compared to the control lambs with 100 mg Zn./Kg. diet. Nevertheless, the deficient lambs continued to maintain a small gain in weight each week for fifteen

weeks. This result, however, did not agree with the abrupt cessation of weight gain observed in lambs and calves fed 1.2 mg Zn./Kg. diet in experiments by Mills et al. (1967). When the dietary Zinc was increased from 1.2 - 3.0 mg Zn./Kg. diet, in experiments by Mills et al., a small gain in weight occurred each week which compared favourably with results reported by Ott et al.; this was despite the fact that the lambs remained Zinc deficient, relative to controls, and showed the accepted external symptoms of deficiency.

Mills et al. (1967) suggested that 7 mg Zn./Kg. diet was sufficient to maintain growth, whilst 15 mg Zn./Kg. diet was required for maintaining the level of blood constituents and overall health.

There is little agreement amongst research results on an optimum concentration of dietary Zinc which can prevent Zinc deficient symptoms. In addition to the dietary Zinc levels suggested by Mills et al., however, Arora et al. (1968 a.) found that 50 mg Zn./Kg. diet was necessary in lambs and calves in order to maintain health and production. Other workers, Underwood and Somers (1969), and Holod et al. (1969) suggested that between 33 and 74 mg Zn./Kg. diet was necessary for maintaining growth in lambs.

Very little relationship exists when the Zinc requirement of ruminants on semi-synthetic diets are compared with the Zinc intake of ruminants that have developed Zinc deficiency under natural occurring conditions. Legg and Sears (1960) found that cattle developed symptoms when consuming grass which contained 18 to 22 mg Zn./kg.

diet in one instance, and 34 to 42 mg Zn./Kg. diet in another instance. Zinc deficiency symptoms have been found in cattle consuming natural diets with even higher Zinc contents. An example was seen by Grashius (1964) who reported that external symptoms of Zinc deficiency were apparent in calves consuming hay, grass or silage which contained 80 mg Zn./Kg. diet. Other examples of ruminants showing external symptoms of Zinc deficiency whilst they consumed natural diets with high Zinc content, have been reported by Hyppola (1966), Haaranen (1963), and Holod (1969). Further review and discussion of these examples are beyond the scope of the present review; such studies involve the interrelationship of Zinc with other constituents of natural diets which are not contained in semi-synthetic diets, and thus relate to Zinc uptake and retention in the animal.

In addition to the external symptoms and the depressed growth rates, many reports claim that decreased food consumption accompanies Zinc deficiency in many animals. These reports, including those by Miller et al. (1968) in pigs, Miller and Miller (1962) in calves; Miller, Pitts and Clifton (1964) in goats, and by Ott, Smith, Stob and Beeson (1964) in lambs, all show that reduced feed intake in Zinc deficient animals is highly characteristic and is more than a Zinc effect of appetite.

In Zinc deficient pigs Miller et al. (1968) found that growth rates diminished before food intake was reduced. Miller (1970) and (1971) has suggested that biochemical or physiological changes or both may precede

the reduced feed consumption in Zinc deficiency. He did not, however, elaborate further on these suggestions, and no further research on this problem has since been published. Further, Mills et al. (1969) reported that forced feeding of Zinc deficient animals caused death; this seemed to demonstrate that Zinc deficient animals were unable to metabolize normal amounts of food.

An interesting result of increased food intake was found, however, by Neathery et al. (1973) in Zinc deficient male goats. They fed Zinc deficient diets to goats for a much longer period, 20 weeks, than previously published for Zinc deficiency experiments. Their results showed that the food intake of deficient goats decreased drastically during the first two weeks and remained depressed for a total of eight weeks. Subsequently, however, the food intake increased steadily until by the eighteenth week of deficiency, the food intake had returned to starting values.

The most interesting fact about this result is that the external clinical symptoms continued to increase in severity whilst the food intake was increasing. Further, there were no compensating improvements in weight of the goats, thus indicating that the food intake was inefficiently used.

It would seem reasonable to suggest therefore that, in goats fed Zinc deficient diets for long periods, increased metabolism of food may occur. Research in this field has not advanced sufficiently to offer a biochemical explanation for this observation.

The importance of the characteristic that animals on Zinc deficient diets for up to ten weeks have reduced food intakes, was brought to light by Mills and Chesters (1970) at the World Association for Animal Production International Symposium. They argued that it was inappropriate in nutritional and metabolic experiments with Zinc to use ad lib. fed control animals as the sole basis of comparison with Zinc deficient animals. Their argument was based from results which showed that both Zinc deficient ruminants and rodents had poor food consumption. After making reference to work by Humphries and Quarterman (1968) on the role of Zinc in appetite regulation, they recommended that the usual recourse should be a paired-feeding regime. This requires that the pair-fed control animals consume restricted quantities of food based on the quantity consumed by the Zinc deficient animals. They also suggested that ad libitum fed control animals are observed in parallel with the pair-fed animals.

In determining the dietary Zinc requirement of various animal species, gain in body weight has been the most frequently used criterion. This was undoubtedly due to the fact that attempts to relate tissue Zinc concentrations to alterations in the intake of this metal with ruminants by Miller and Miller(1962), and Ott, Smith, Stab and Beeson (1964) and with rats by Miller, Fischer, Elcoate and Mawson (1958) had shown that, with the exception of the pancreas, muscle, brain and possibly bone, there was little change in the Zinc content of body tissues, this was true whether the diet was Zinc deficient or contained an

excess. This indicated the possibility, therefore, that some tissues may possess homeostatic mechanisms for Zinc control.

In these studies of determining the effect of Zinc deficiency on the Zinc content of tissues, few reports have examined the Zinc content of blood. Those that have, find it to be relatively constant, despite the low dietary intake of the element, and the onset of clinical symptoms. In experiments by Miller and Miller (1962), clinical signs of Zinc deficiency in calves developed in eleven weeks, whilst Zinc in whole blood fell by only 30 per cent from 2.7-1.9 µg/ml. This contrasted with results by Mills et al. (1967) where clinical signs of Zinc deficiency in calves developed in only two weeks, when the Zinc in plasma fell more dramatically than in blood, by 78 per cent from 0.9 - 0.2 µg/ml. The calves in both sets of experiments had approximately the same daily zinc intake. Similar rapid decreases in plasma Zinc levels were found for Zinc deficient lambs by Mills et al. (1967). In contrast, the plasma Zinc levels of Zinc deficient lambs in experiments of Ott et al. (1964) did not show rapid decreases, and took ten weeks to reach levels of plasma Zinc similar to those in experiments of Mills et al.

The results of studies by Mills et al., showed that if the plasma concentration of Zinc decreased below 0.4 µg/ml and remained below this figure for more than one week, then growth was arrested and later, clinical signs of Zinc deficiency developed. However, during the first week on Zinc deficient diets, good rates of growth were maintained, even when plasma Zinc concentrations were low. This

suggested that plasma Zinc determinations may have a limited value for the detection of Zinc deficiency and also the possibility that homeostatic mechanisms for controlling physiologically available Zinc may be present in plasma.

The external clinical symptoms of Zinc deficient animals has been correlated with the activity of alkaline phosphatase in the plasma. This enzyme is, however, only one of several enzymes which require Zinc as an essential component for activity. Early workers concerned with Zinc deficiency such as Hove, Elvehjam and Hart (1940) found that the activity of intestinal alkaline phosphatase was decreased in Zinc deficient rats. Similarly, Day and McCollom (1940); Luecke, Olman and Baltzer (1968); Newland, Ullery, Holfer and Luecke (1958), and Oberlas et al. (1966) all found that alkaline phosphatase activity decreased in plasma of pigs fed Zinc deficient diets. Moreover, they found that the reduced enzyme activity occurred noticeably at the same time as the external clinical symptoms.

Earlier work by Luecke et al. (1956) in pigs, and later by Miller (1965) in calves, showed that the level of this enzyme activity in plasma was correlated with the degree of Zinc deficiency. Thus the lower the activity, the greater was the severity of the skin lesions. Moreover, Kirchgessner and Schwartz (1975) have shown that serum alkaline phosphatase can reflect the mobilization of endogenous Zinc reserves in lactating cows by showing increased enzyme activity; therefore they suggest the activity of this enzyme is the most suitable measurement

for diagnosis of Zinc deficiency. Several workers, including Fujioka and Lieberman (1964), Williams et al. (1965), Buchanan and Hsu (1968), and Williams and Chesters (1970) have investigated the effects of Zinc deficiency on the synthetic rates of protein and D.N.A. The general concensus which emerged was that Zinc deficiency caused marked inhibition of protein and D.N.A. synthesis. This would suggest that a change in Zinc status of an animal might be reflected in the concentration of plasma proteins. Despite this possible association and its far reaching implications, very few reports have been published of work investigating plasma protein levels in Zinc deficient animals.

A decrease of total plasma proteins has been reported by Hove et al. (1940) in rats fed Zinc deficient diets and more recently this decrease was supported by results by Shyy-Hwa and Hurley (1971). They found a significant ($P < 0.01$) reduction in total plasma proteins of rats after four weeks of consuming Zinc deficient diets. Similar reductions in plasma protein concentrations have been found by Rahman et al. (1961) in chicks and by Fox et al. (1965) in Zinc deficient quail.

By contrast, the total plasma proteins were found to increase significantly ($P < 0.01$) when Zinc deficient diets were fed to lambs in experiments by Ott et al. (1964) and to pigs by Miller et al. (1968).

Further analysis and measurement of the plasma protein fractions in Zinc deficient pigs were made by Miller et al. (1968a); these showed that whilst the pigs continued to consume the Zinc deficient diet, the total

globulin level increased. This, however, was entirely due to a significant ($P < 0.01$) increase in the concentration of γ and α_2-globulins. Moreover, the albumin concentration in these pigs, decreased significantly ($P < 0.01$). These results confirm earlier results in pigs by Hoefer et al. (1960) and by Smith et al. (1960). Although Ott et al. (1964) attempted similar estimations of plasma protein fractions in Zinc deficient lambs, their results were inconclusive because inadequate methods of fractionating the globulin fraction had been carried out. However, their results showed a significant ($P < 0.01$) increase in the total globulin concentration, which they suggested was probably a response to secondard infection from the open lesions on the skin of the Zinc deficient lambs. The albumin concentration in these lambs showed a tendency to decrease but the difference in levels was not significant.

Further evidence of changes in protein concentration in Zinc deficient lambs was observed by Parry and Lacey (1975), when the plasma fibrinogen level increased significantly ($P < 0.01$) during six weeks of Zinc deficiency.

If further understanding of Zinc nutrition in ruminants is to be achieved, it seems reasonable to assume that investigations on the distribution, binding and transport of Zinc in plasma must be pursued. Such work might be expected to throw light on the homeostatic mechanisms which provide ionic or physiologically available Zinc in a suitable form for biochemical activity, and also on the relationship of ionic to bound Zinc. It would be expected that changes in the ratio of ionic to bound Zinc might also change in Zinc deficiency and be responsible for

the appearance of clinical symptoms.

Studies on the binding of Zinc to plasma proteins were first made by Vikbladh (1951). Subsequently, more extensive studies were made by Wolff (1956) and Parisi and Vallee (1970). The results from these experiments have shown that Zinc in human plasma is transported by plasma proteins partly as exchangeable or loosely bound Zinc, and partly as non-exchangeable or firmly bound Zinc. These two forms of protein bound Zinc correspond to the metal-protein complex and metalloprotein fractions respectively of Vallee and Wacker (1970). They based this distinction on the strength of the chemical linkage between the metal and the associated protein. In metalloproteins, the metal atoms are bound very firmly and do not separate from the protein during analytical procedures; therefore the protein would ultimately, after analysis, contain stoichiometric quantities of metal. In metal-protein complexes, the metal atom is loosely bound and its linkage with the protein is more tenuous. Vallee and Neurath (1954) and (1955) found that on progressive purification, the ratio of metal to protein, and metal to enzymic activity and of enzymic activity to protein approached fixed limits. The loosely bound metal ions, which were not related to enzymic function, were removed as purification was achieved, and their concentration eventually decreased to negligible values.

The identification of metal-binding sites of proteins has been based largely on the mode of interaction of metals with amino acids, peptides, and their derivatives. Such studies described by Vallee and Wacker (1970) have led

to the general conclusion that amino acid side chains of proteins, having dissociable hydrogen ions, serve as the ligands for metal interactions; though peptide nitrogen atoms can also participate as shown by Martin and Edsall (1960) and Koltun et al. (1963).

Almost all of the potentially reactive groups of amino acids have either been demonstrated or postulated by Vallee and Williams (1968a) to bind metals; these groups include the α-carboxyl and aspartic acids; the ϵ-amino group of lysine; the imidazole group of histidine; the phenoxy group of tyrosine; sulphydryl groups of cysteine and the guanidinium group of arginine.

Metalloproteins are distinguished by the small number of metal ions which are firmly bound to the proteins. These metal ions according to Vallee (1960) are bound to a limited number of specific sites on the protein molecule. Vallee and Williams (1968 a,b.) suggested that in general a metal prefers a particular arrangement of the ligand groups with which it interacts.

This metal ion binding has been argued to cause conformational changes in proteins which can be detected by X-ray, optical rotatory and spectrophotometric studies. Such measurements by Gurd and Murray (1954) and Perkins (1961) in metal containing proteins suggest that the probable site of Zinc binding is to the side chain carboxyl groups and possibly to the histidyl side groups mentioned by Gurd and Goodman (1952), and Edsall et al. (1954 a,b.).

Earlier reference was made to plasma Zinc being bound either loosely or firmly to plasma protein; the metal-protein complex and metalloprotein fraction

respectively. The loosely bound Zinc was recognised by Wolff (1956), Prasad (1966), and Parisi and Vallee (1970), when isotopic Zinc was added in vitro to human plasma. They isolated and identified albumin as the plasma protein which contained the loosely bound Zinc. Further, in vitro and in vivo analysis showed that the Zinc content of albumin was freely exchangeable with $^{65}Zn.$; therefore they concluded that Zinc in albumin was loosely bound.

Independent biochemical studies by Kägi and Vallee (1960) on the metal binding properties of albumin and its reactive groups have suggested the possibility of Zinc binding to thiol (-SH) groups. These studies have also suggested that albumin may be heterogenous. Human and bovine plasma albumin was freshly prepared by ammonium sulphate precipitation by Edsall et al. (1954 a) and its molecular weight determined as 68,000. This albumin contained less than one thiol (-SH) group. The thiol content of the fresh human albumin was within the range 0.65-0.70 -SH groups per mole and that of bovine albumin was within the range 0.50-0.75 -SH groups per mole. Although albumin preparations were found to be homogenous by ultracentrifugation and electrophoresis, data by King et al. (1960) however, suggests the existence of two types of albumin molecules. They found that one group of albumin molecules contained a thiol group, whilst the other group contained mixed disulphides.

The possibility that albumin molecules may exist in two types raises doubt whether all the Zinc in albumin is distributed between the two types with similar loose bonding referred to earlier. The answer to this problem

might affect the availability of Zinc in nutritional disorders of animals.

Work reported by Shrivastava, Goch and Zakrzewski (1972) showed that albumin molecules could be fractionated into three major fractions by chromatography and electrophoresis. They expressed some doubt whether the observed heterogeneity of human albumin might reflect the in vivo state, or alternatively whether it is an artifact caused by preparative and analytical procedures, as has been suggested by Andersson (1966).

The amount of Zinc bound to human albumin has been determined by several workers, but uncertainty exists as to the proportion of the total plasma Zinc which is bound. It is necessary to refer to human plasma sample analysis because similar Zinc binding studies have not been published for ruminants.

Sixty to seventy per cent of total plasma Zinc was found by Prasad (1966) to be loosely bound to albumin. Giroux and Henkin (1972) however found that a higher proportion, 85-98 per cent of total plasma Zinc was bound to albumin.

Evidence is available to show that Zinc is bound to other plasma proteins in addition to albumin. One of the earliest reports to suggest this possibility was made by Vikbladh (1951), who suggested that Zinc was bound in a specific way to 'special' carrier-proteins. His results suggested that whilst all human plasma Zinc was protein bound, 35 per cent was tightly bound to a globulin protein and the remainder was loosely bound to albumin.

Later, Wolff (1956) drew attention to the importance of Zinc being bound to a number of plasma proteins. He suggested that 60 per cent of human plasma Zinc was loosely bound. He did not, however, make any further suggestions on the properties of globulins as Zinc binding proteins. Vessel and Bearn (1957) suggested that Zinc was bound to α_2-globulin.

Further investigations on the Zinc binding globulin did not advance until analytical techniques in metal and protein analysis had progressed. As plasma Zinc is at such low concentrations it was not until 1960 when the sensitive and accurate technique of atomic absorption spectrophotometry was developed that accurate measurements could be obtained. Furthermore, the methods of protein separation used prior to 1956, involved conditions which were disruptive to metal-protein studies. However, advances were made when Peterson and Sober (1956) and Sober et al. (1956) developed their technique of ion-exchange chromatography for seperating plasma proteins with high resolution at any required sample load.

The adaptation of these techniques by Himmelhoch et al. (1966) and later by Parisi and Vallee (1970), resulted in the separation from human plasma of the metalloprotein, α_2-macroglobulin. This protein was found to contain firmly bound Zinc. Himmelhoch et al. (1966) fractionated human plasma proteins by using ion-exchange chromatography and determined Zinc by atomic absorption spectrophotometry. Three Zinc containing fractions were isolated. The Zinc content of these fractions were non dialysable thus indicating that the element was firmly

bound. They did not, however, attempt to purify these fractions. Parisi and Vallee (1970 modified this method of fractionation by introducing 'gel filtration' into the procedure and by minimizing metal contamination. Therefore Parisi and Vallee were able to isolate α2-macroglobulin and analyze its content of bound zinc. This protein molecule was shown to bind 30-40 per cent of the total Zinc of human plasma. Although they did not estimate the amount of Zinc in albumin, they nevertheless tested both the α2-macroglobulin and albumin fractions to see whether the stable Zinc in these fractions was exchangeable with ^{65}Zinc. The result showed no ^{65}Zinc in the α2-macroglobulin fraction but a substantial amount was recovered from the albumin. Thus the α2-macroglobulin bound Zinc was firmly bound, in contrast to the loosely bound Zinc in albumin. They estimated that the mean quantity of bound Zinc in α2-macroglobulin was between 320-770 µg/g of protein, corresponding to 4.1-10.0 g.atoms of Zinc/mole.

Zinc has been found bound to other protein fractions and amino acids in human plasma, in addition to albumin and α2-macroglobulin by Prasad and Oberleas (1970), Boyett and Sullivan (1970), and Giraux and Henkin (1972).

The results reported by Boyett and Sullivan (1970) showed that a mean total plasma Zinc concentration of 75.5 ± 12.7 µg/100 ml was bound more or less completely by three fractions: albumin bound 63.5 ± 10.6 µg. Zn./100 ml, γ-globulin bound 1.6 ± 0.7 µg. Zn./100 ml, and a third fraction, Tf-α2-macroglobulin binding 11.5 ± 2.4 µg. Zn./100 ml. This third fraction was composed of transferrin and α2-macroglobulin plasma proteins. A reciprocal

relationship between the amount of Zinc bound to these proteins was found; when the Zinc concentration was high in transferrin it was low in α2-macroglobulin and vice versa.

The importance of amino acids as binding molecules for Zinc in plasma was brought to light by the work of Prasad and Oberleas (1970). Using in vitro studies of separated human plasma proteins and amino acids, they found that amino acids competed effectively with albumin, haptoglobulin, transferrin and IgG for the binding of Zinc in solution. A similar phenomenon was not observed with ceruloplasmin and α2-macroglobulin; these two proteins showed special stronger binding properties with respect to Zinc in vitro.

Of the many amino acids which they studied, histidine was most prominent in its binding properties for Zinc; Gurd and Goodman (1952) have reported previously that Zinc ions are bound to the imidazole group of the histidine residues in the albumin. Free amino acids are present in plasma and thus it is likely that some plasma Zinc which is not bound to plasma proteins may be loosely bound to these amino acids. This amino acid-bound fraction of plasma Zinc may have an important role in the absorption and transport of this element from the gastro-intestinal tract.

This, however, may not be the only method of transport in portal blood as demonstrated by Evans and Winter (1975) in the rat. They found that in portal venous plasma, whilst the major fraction of ^{65}Zinc was

bound to albumin the remainder of the isotope was associated with higher molecular weight proteins including transferrin and α2-macroglobulin.

Very little information is available in the literature which gives an estimate of the amount of plasma Zinc which is not bound. Prasad and Oberleas (1970) estimated the amount of unbound human plasma Zinc by ultrafiltration as 2-8 per cent of the total plasma Zinc.

Considerable attention has been given in this review to the identity of the binding proteins and the amound of Zinc which they bind as a proportion of the total Zinc in normal human plasma. Such studies are important in that they may throw light on possible mechanisms controlling the availability of Zinc for maintaining physiological functions in the normal as well as in Zinc deficient conditions.

In humans the effect of Zinc deficiency on plasma bound Zinc has not been fully investigated. The same is true for ruminants or other animals. Low plasma Zinc has been observed as a symptom in some diseases.

In patients suffering with Laennec's cirrhosis, where the total plasma Zinc was low, Boyett and Sullivan (1970) found that the amount of Zinc bound to Tf-α2-macroglobulin remained constant, even when the total plasma Zinc fell by 41 per cent. The albumin bound Zinc, however, was shown to decrease linearly with total plasma Zinc, thus indicating that this Zinc was loosely bound and more labile in contrast to the more firmly bound Zinc to Tf-α2-macroglobulin.

The amount of Zinc bound to albumin in Zinc deficient plasma was unrelated to that amount bound to Tf-α2-macroglobulin. It is possible, however, that the total plasma zinc was not sufficiently low to cause a change in the amount of Tf-α2-macroglobulin Zinc.

These results suggest that the Zinc binding to Tf-α2-macroglobulin is a constant amount, and available possibly even in extreme Zinc deficient conditions to satisfy metabolic essentials.

Zinc homeostatis in mammals was first reported by Borg and Cotzias (1958) in the rat. Later, Vallee (1959) observed that the concentration of Zinc in several tissues remained relatively constant in Zinc deficient rats, thus indicating homeostatic regulation.

Two methods of homeostatic control of Zinc were postulated by Cotzias, Borg and Selleck (1961) and Cotzias and Papavasiliou (1963); the first was the control of Zinc excretion and the second method was by controlling the absorption of the element.

Both methods of control of Zinc levels were shown to occur in calves by Miller et al. (1966a), (1968), (1970) and (1971). Of the two controls changes in absorption, however, appeared to be a greater factor. Absorption values for ^{65}Zn were observed as high as 80 per cent in calves fed low Zinc diets containing 2 - 4 mg Zn/Kg. diet compared with 53 per cent in calves fed a non Zinc deficient diet containing 35 mg Zn/Kg. diet.

Whilst Miller (1970) and Miller et al. (1970) were unable to show the breakdown of this mechanism for Zinc absorption in a very severe deficiency state,

increasing dietary Zinc to high levels (Miller et al. (1971)) caused the mechanism to begin to break down. This was seen by the Zinc content of some tissues, which had maintained constant Zinc levels in Zinc deficiency, increasing in total Zinc content; this increase was far greater than the proportionate increase in total Zinc intake, thus indicating selective accumulation and homeostatic breakdown. Whilst the Zinc intakes were high, they were nevertheless substantially below those associated with any gross indication of toxicity.

On the whole the data available supports the concept that the percentage of dietary absorption and endogenous excretion of Zinc is dependent on tissue levels of Zinc and thus tends to maintain homeostatic control of Zinc at the tissue level. Miller (1969) suggests that the changes of Zinc level in soft tissues is somehow reflected back to the intestine where the Zinc absorption and endogenous excretion occur. Miller et al. (1966), (1968), have shown that feeding a Zinc deficient diet to calves had two effects on tissue Zinc levels. The first effect was a decrease in Zinc content of many tissues within a few days after consuming the diet; this decrease was not large and it varied between the tissues. The second effect lasted over the subsequent few weeks when the level of Zinc in these tissues did not appear to change; if the feeding of Zinc deficient diet was continued long enough, external symptoms of deficiency became apparent and a reduction of Zinc occurred in some of these tissues whilst in the others, the Zinc level remained reasonably constant.

It has been suggested that several tissues possess their own control of Zinc concentration. When Zinc deficient diets were fed to pigs by Cassens et al. (1963) and to cattle by Miller et al. (1965) and Miller (1970), Zinc content of some tissues, including liver, pancreas, kidney, hair and bone was found to decrease. In other tissues, including brain and muscle, however, the zinc content remained unaffected, even when external symptoms of deficiency were apparent. This would appear to limit severely the usefulness of tissue Zinc level in diagnosis of a Zinc deficiency in individual animals, a fact previously recognised by Blackmon et al. (1967).

This evidence suggests that in Zinc deficient animals, some individual tissues have their own homeostatic mechanisms for controlling Zinc levels.

The exchange rate of Zinc from blood to tissues such as liver, kidney, heart and lungs was found by Miller (1969) to be much more rapid than to tissues such as red blood cells, bone, muscle and hair. This, therefore, suggests that blood plasma has two forms of bound zinc, a loosely bound Zinc for efficient exchange and supply to the tissues, and a more firmly bound Zinc. The effect of Zinc deficiency on bound plasma Zinc has not been fully investigated in lambs.

CHAPTER 3

Aims of Experimental Work

It is established that ruminants, similar to mammals in general, require adequate dietary Zinc for normal health and functioning. In deficiency of Zinc however, research has shown that pathological, behavioural, physiological and biochemical symptoms are evident. There are also alterations in general performance of animals. The results of studying these various symptoms have been inadequate, largely because each symptom has frequently been studied in isolation of other symptoms, with little attempt to relate the results of such study to the animal as a whole.

In general, homeostasis of elements is the ability of the animal to maintain constancy with varying intake levels. Accordingly, investigations of factors influencing homeostasis of Zinc, such as severity of Zinc deficiency, and the mechanism of Zinc transport, would consider both fundamental and applied aspects of biochemistry and nutrition.

It was the intention of this thesis therefore, to examine whether experimental evidence was available for detecting homeostatic control of Zinc in lambs which were fed a severe Zinc deficient diet. To fulfil this aim, the investigations considered the effect of dietary Zinc deficiency on:

(a) performance,

(b) plasma proteins,

(c) bound and unbound Zinc in plasma.

In addition to this applied approach, it was anticipated that fundamental knowledge on the binding of Zinc to plasma proteins in lambs would be advanced.

It was considered necessary in these investigations to use both male and female lambs. The reason for this was based on suggestions that steroid hormones may influence the concentration of Zinc in plasma. McBean, Smith and Halstead (1971) and Halstead, Hackley and Smith (1968), found that oestrogens in the human female lowered the plasma Zinc without causing a total body depletion of the element. Aitken, Lindsay, Hart and McKay (1973), later confirmed this result in human females, and further suggested that progesterone was also involved. Flynn et al. (1973) found that an injection of progesterone into human males, increased the concentration of plasma Zinc. More recently, Yunice et al. (1975) has confirmed these results and suggested that a balance could be operating in females between oestrogens causing a decrease in Zinc, and progesterone causing an increase in plasma Zinc. This further suggests that the female might have a greater advantage of homeostatic control of Zinc over the male. This could lead to the possibility that the female is more protected against Zinc deficient effects.

Six experiments were planned and carried out. The results of each one are shown in Chapters 5 to 10. Whilst the main aim of these experiments has been stated above, the individual aims and the co-ordination between these experiments are described below.

In Chapter 5 there were two aims. The first was to see whether dietary Zinc deficiency affected three criteria of performance of lambs. These criteria were growth, food intake and food conversion. This experiment differed in two respects from the work of Mills et al. (1967); firstly by its design, and secondly by the low dietary Zinc content of 0.9 mg Zn/Kg. diet. The second aim was to see whether these criteria would reflect any mobilization of endogenous Zinc reserves under severe dietary Zinc deficiency.

In Chapter 6 the aim was to measure the activity of the Zinc dependent enzyme, plasma alkaline phosphatase with the intention of comparing this activity with external clinical symptoms of Zinc deficiency observed by Day and McCallom (1940), Luecke et al. (1956), and Miller et al. (1965). Their results showed that a reduction in plasma alkaline phosphatase activity in cows and pigs was closely related to the severity of external clinical lesions.

In Chapter 7, the aim was to investigate whether a Zinc deficient lamb maintains its plasma protein concentration in total or the concentration of some individual fractions. This experiment was planned in the light of the results from Chapter 5 which showed that the growth rate of Zinc deficient lambs continued to increase, but at a much reduced rate than in control lambs. There is abundant evidence by Pories et al. (1967), Buchanan et al. (1968), Mills et al. (1969) and Williams and Chesters (1970), that Zinc is necessary for protein synthesis and cell growth. This evidence coupled with the result in Chapter 5, suggested

that physiologically available Zinc might have been used for protein synthesis enabling the maintenance of minimum growth noted in Zinc deficient lambs. The level of plasma proteins might reflect the activity of protein synthesis and, in turn, the physiologically available Zinc. Experiments were designed to measure the concentration of plasma proteins and the individual fractions:- albumin, total globulin, $\alpha 1$-, $\alpha 2$-, β, and γ-globulins. In this way, evidence might be found that a homeostatic control mechanism exists, which maintains physiologically available plasma Zinc concentration under severe dietary Zinc deficiency. The experiment was therefore intended to investigate the concentration of plasma proteins and individual protein fractions in Zinc deficient, and subsequently Zinc supplemented male and female lambs.

In Chapter 8, there were two aims. The first was to obtain an estimate of the amount of Zinc bound to plasma proteins in lambs fed a Zinc supplemented diet; the second aim was to investigate whether a Zinc deficient diet had an effect on the amount of bound and unbound plasma Zinc. Previously, Haurowitz (1961) and Vallee (1960) showed that Zinc was bound to human plasma proteins, but no work has been found published for Zinc bound to lamb plasma proteins. The first aim of this experiment was concerned with Zinc bound to proteins of molecular weight greater than 500. From this result, anticipating that some Zinc was protein bound, the quantity of unbound Zinc could be calculated by subtracting the amount of protein bound Zinc from the total plasma Zinc.

It is well recognised - Hambridge (1974) - that the total Zinc concentration in plasma and tissues, as with several other elements, is not always related to the amount of that element which is physiologically available. This quantity of an element is difficult to assess. The terms 'diffusible', 'non-protein bound', 'ionized', 'free', and 'ultrafiltrable' have been used interchangeably in the literature, and it is recognised that they can represent different plasma constituents. It is the intention in these experiments to use the realistic term of 'unbound Zinc'. This will include the amount of Zinc which is physiologically available.

In Chapter 9, it was intended to determine and characterise the plasma proteins which were able to bind Zinc in lambs fed Zinc supplemented diets. This aim was suggested in the light of results from the previous experiment where Zinc was found bound to plasma proteins.

In Chapter 10, the aim was to investigate whether the Zinc bound to the proteins which had been characterised in Chapter 9, was influenced in lambs fed a Zinc deficient diet. This result was a prerequisite to enable the unbound Zinc fraction to be assessed. A further assessment could then be made of the possibility that lamb plasma may have a homeostatic mechanism for the control of physiologically available Zinc.

The identification of metabolic defects arising during dietary Zinc deficiency in these experiments could provide information which may well be applied to the task of improving techniques for the detection of such disorders in the field.

CHAPTER 4

Experimental Methods.

General Experimental Design and Treatments.

Pre-experimental Treatment

(i) Clun Forest lambs, each 6-8 weeks old were purchased between 1971 and 1974 from the University of Bristol farm at Langford House. In 1971 fifteen male lambs and in 1972 fifteen female lambs were purchased for experiments described in Chapters 5,6,7 and 8; the data on these individual lambs is recorded in Appendix 14-23. Additinnal lambs were purchased for experiments in Chapters 9 and 10. Initially all lambs were fed a normal diet consisting of hay and concentrate (Lamb and Ewes pencils from Oxoid Ltd.).

Fourteen days before the commencement of any experiment using the basal Zinc deficient diet the hay and concentrate diet was withheld and all lambs were fed on the Zinc deficient basal diet, described on page — supplemented with $ZnSO_4.7H_2O$; this diet is henceforth called supplemented basal diet and it contained 40 mg Zn/Kg. diet.

(ii) Animal house and pens

The lambs were housed in individual pens throughout the experiments. The pens were made of wood with concrete floors. The metallic fittings were covered by plastic sheets so that lambs could not lick them. Wooden slattings were used on the floor to serve as bedding.

The pens were washed and cleaned three times per week. Temperature of the house was approximately 68°-70°F. throughout and exhaust fans exchanged the air continuously.

(iii) Diets and Water

Two types of diet were prepared - a Zinc deficient basal and a supplemented basal diet.

The preparation of the Zinc deficient basal diet was according to the formulation by Mills et al. (1967) with little modification; it is described on page 43.

This diet contained 0.9 mg Zn/Kg. diet after analysis by atomic absorption spectroscopy described on page 46.

The supplemented basal diet was prepared by the addition of a calculated quantity of Zinc sulphate ($ZnSo_4.7H_2O$) to the Zinc deficient basal diet so that it contained 40 mg Zn/Kg. diet.

Diets were offered in plastic feeders each morning at the same time.

Experimental Treatment

(i) Fifteen lambs were randomised by weight into three comparable groups containing 5 lambs in each. Each group was treated differently as follows:-

Zinc deficient group: fed Zinc deficient basal diet (0.9 mg/kg Zinc) ad lib.

Pair-fed control group: fed restricted quantity of supplemented basal diet (40 mg Zn/Kg. diet). The daily food intake of individual lambs in this group was equated with the previous day's food intake of lambs in the Zinc deficient group.

Ad lib. control group: fed an unrestricted amount of supplemented basal diet (40.0 mg Zn/Kg. diet).

The duration of each experiment was twelve weeks. This time was divided into two period of six weeks.

During Period 1 (Weeks 1-6 inclusive) the groups were:

Zinc deficient group

Pair-fed control group

Ad lib control group

During Period 2 (Weeks 7-12 inclusive) the Zinc deficient and ad lib control groups exchanged treatments, so that:-

Zinc deficient group - ad lib control group in Period 1,

Pair-fed control group - remained on same treatment,

Ad lib control group - zinc deficient group in Period 1.

(ii) Collection of Data and Samples for Analyses

(a) Data for growth and food conversion efficiency

The live weight of lambs was recorded once per week and growth rates were calculated as g live weight gain per week.

The lambs were offered their diet at the same time each morning.

The lambs which were fed ad lib i.e. the Zinc deficient and the ad lib control groups, were allowed to eat to their appetite. The quantity of food uneaten was weighed back the next day, and in this way a 24-hour food intake was recorded.

The individaul lambs in the pair-fed control group consumed the same quantities of food as lambs in the Zinc deficient group.

The food conversion efficiency (F.C.E.) was calculated by the ratio:

$$\frac{\text{Food intake (g/week)}}{\text{Growth rate (g/week)}}$$

(ii) (b) Preparation of Plasma Samples

10 ml of blood from the jugular vein was collected into heparinised tubes twice each week. Plasma was seperated by centrifuging at 3000 r.p.m. for 10 minutes and kept in a freezer at -20^{0}C.

(c) Clinical Observations

The lambs were observed daily for any clinical symptoms of Zinc deficiency on the body surface. Gross clinical symptoms were photographed and are shown in Chapter 6.

(d) Biochemical Estimations

The plasma samples were analysed for:- concentration of Zinc, total protein, alkaline phosphatase activity, and protein fractions which bound Zinc.

Methods of analyses are described seperately.

(e) Statistical Analysis

The factorial method of analysis of variance was used. Analysis was carried out on the last four weeks of each six week period. This gave lambs two weeks of adjusting to new diets at the beginning of Period 2.

Statistical significance of treatment differences were determined by the F test and the multiple range test of Duncan (1955).

Preparation and Composition of the Zinc Deficient Basal Diet

The diet was prepared from the ingredients shown in Table (i).

The mixing procedure was carried out as follows. Four seperate premixes were prepared. The first, premix A, contained all vitamins and antibiotics shown in Table (iii); the second, premix B, containing NaCl and trace elements (Fe; Cu; Mn; K) shown in Table (ii); the third premix C, contained the other minerals shown in Table (ii); the fourth, premix D, with the exception of choline chloride and Arachis oil, contained the other components in Table (i).

Spray-dried egg albumin was obtained from Messrs. H.D. Hardie and Co., Edinburgh, Solka floc-B.W.40 cellulose from Johnson, Jörgenfen and Wettre Ltd., and maize starch from Corn Products Corporation (C.P.C.). All other ingredients of the diet shown in Table (i) were supplied by Dalgety-Crosfields Ltd.

Each premix was seperately mixed in a stainless steel mixer for fifteen minutes.

Premixes A, B and C were added to Premix D and mixed for fifteen minutes. Choline chloride mixed with some of the sucrose was then added to the total mix, and mixed for a further five minutes. This procedure was carried out because choline chloride is a water absorbent substance and could not be mixed directly with the minerals. Finally Arachis oil was added with further mixing of all the dietary components for ten minutes.

The diet was stored in polythene bags. Before each batch of this diet was fed to the lambs, a sample

was analysed for Zinc content. During the series of experiments the Zinc content of the diet ranged between 0.75 mg and 1.20 mg/Kg; mean 0.9 mg Zn/Kg. diet.

With the co-operation of Dalgety-Crosfields Ltd. for frequent supply of diet ingredients and the mixing facilities at the Veterinary School, University of Bristol, Langford, the diet was prepared in batches. Each batch was sufficient food supply for lambs for six weeks.

Table (i)

Percentage Composition of the Zinc Deficient Basal Diet

Ingredients	% of Diet
Dried egg albumin	9.00
Glucose	32.00
Maize starch	38.00
Solka Floc Cellulose	9.00
Arachis oil	6.00
Mineral supplement	5.40
Vitamin and antibiotic supplement	0.56
Choline chloride	0.14

Table (ii)

Composition of 100.00g mineral mixture

Ingredients	Quantity (g)
$CaHPO_4.2H_2O$	40.92
$CaCO_3$	4.08
$Mg\ SO_4.7H_2O$	17.12
$KHCO_3$	14.26
Na Cl	22.97
$Fe.SO_4.7H_2O$	0.28
$Cu\ SO_4.5H_2O$	0.04
$CoCl.6H_2O$	0.01
$Mn\ SO_4$	0.30
Kl	0.23×10^{-3}

Table (iii)

Vitamin and Antibiotic Premix (mg/kg of diet)

Ingredient	Quantity
Biotin	0.22
Naphthoquinone	2.20
Pyridoxin	2.20
Folic acid	4.40
Thiamin hydrochloride	6.60
Riboflavin	8.80
Vitamin A (362000 lu/g)	12.21
Ca-pentothenate	22.00
Niacin	33.00
Vitamin E	33.00
Vitamin B_{12}	44.00
Ascorbic Acid	110.00
1 innositol	110.00
Vitamin D (Delestrol 3000 i.u./g)	14.30
Chlorotetracycline (Aureomycine)	22.00

Determination of Zinc in Plasma

A modification of the method suggested by "Unicam" Instruments Ltd. (Method Sheet Zn3) Atomic absorption spectroscopy was adopted for the determination of Zinc in plasma.

All glassware was immersed in 2M.HNO_3 over night and rinsed thoroughly with de-ionised water.

Method

Plasma was obtained as described earlier. A dilution of 1:3 with de-ionised water was made and the optical density of the sample was measured at 213.9 nm in a Pye Unicam SP.90 atomic absorption spectrophotometer. An oxygen/acetylene flame and a slit width of 0.15mm were used. The Zinc concentration in the diluted plasma samples was read against a standard curve and the value multiplied by the dilution factor to obtain the plasma Zinc concentration. The calibration curve was checked by standards after every tenth plasma Zinc concentration was determined.

Reagents

(i) Stock zinc solution, 100 mg/L

0.1 g of pure zinc was dissolved in a minimum quantity of concentrated Analar Hydrochloric Acid and made up to 1 litre with de-ionised water. It was stored in a polythene bottle.

(ii) Stock zinc solution, 10 mg/L

10 ml of the stock zinc solution (i) was pipetted into a 100 ml acid washed volumetric flask and made up to the mark with deionised water.

Calibration

Suitable dilutions of the stock Zinc solution (ii) with deionised water were made so that standard solutions could be made up containing 5, 2, 1, 0.8, 0.6, 0.4, 0.2 and 0.1 mg Zn/L.

Determination of α2-Macroglobulin

This method is based on the ability of α2-macroglobulin to bind to trypsin, thereby forming an enzymatically active complex (Schultze and Heremans, 1966). In the complex, the esterase and peptidase activities of trypsin are protected from inactivation by soybean trypsin inhibitor. The amount of complex formed and, therefore, the degree of protection of trypsin activity, is proportional to the amount of α2-macroglobulin present (Haverback et al, 1962; Mehl et al, 1964).

The trypsin-protein esterase activity was measured titrimetrically, using 0.01 M tosyl-DL-arginine methyl ester (TAME, Sigma Chemical Co.) in 0.001 M Tris-Cl, pH 7.5, as substrate, 0.08 mg twice recrystallised bovine trypsin (Sigma Chemical Co.) and 0.10 mg soybean trypsin inhibitor, (Sigma Chemical Co.).

0.2 ml of the fraction was taken with 0.2 ml of trypsin and 0.2 ml of soybean inhibitor, in a total volume of 0.6 ml. After 5 minutes of incubation at 25°C, 0.3 ml of this mixture was added to the substrate (2.7 ml) to start the reaction. The assay was performed at 25°C, PH 7.5, titrating with 0.1 M NaOH in a pH-stat with an automatic recorder. One unit of trypsin-protein esterase activity ≡ 2.0 μmoles of H^+ released per minute.

Separation of Plasma Proteins By Ion Exchange Chromatography

Plasma samples were obtained as previously described. Prior to chromatographic procedures each plasma sample was dialysed against 200 ml of starting buffer, PH 8.6, 0.005 M Succinate and 0.04 MmTris - for 24 hours at 4°C.

The plasma samples were chromatographed on a 2.6 x 40 cm (Pharmacia Ltd.) column of D.E.A.E. - cellulose, micro-granular (Whatmans D.E.32). The method adopted was similar to Parisi and Vallee (1970) with modifications suggested originally by Himmelhoch et al (1966).

The ion-exchanger, D.E.A.E.-cellulose was precycled according to the manufacturers' instructions - Whatmans Instruction Manual 1973. This allowed the cellulose to swell in a repeatable manner. All reagents used were were Analar grade.

The ion-exchanger slurry was packed into the column and washed with buffer - 0.025M Succinic acid and 0.2 M Tris - until the conductivity and pH of the effluent were constant. This buffer, which was five times the concentration of the starting buffer, was pumped through the column at 250 ml per hour.

The packed column was then washed with starting buffer 0.005 M Succinate and 0.04 M.Tris - at 90 ml per hour. The conductivity and pH of the effluent were monitored after every column volume of buffer had passed through to ensure complete equilibrium. The conductivity and pH of the effluent were 1.4 milli mho and 8.6 respectively when equilibrium was first established.

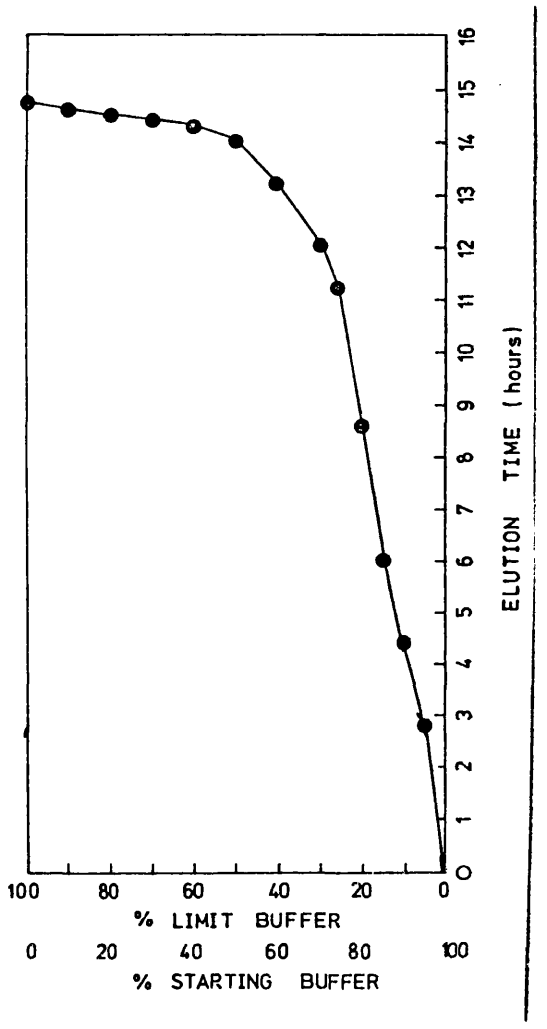


FIG. 1 GRADIENT OF PER CENT LIMIT BUFFER AND PER CENT STARTING BUFFER

After the dialysed plasma sample had been applied to the top of the column and washed in, the column was connected to a peristaltic pump already adjusted to give 70 ml/hour flow rate. 10 ml fractions were collected in an LKB fraction collector. The limit buffer was 0.2 M Succinate and 0.04 M.Tris. A total of 900 ml buffer was used. The gradient of % limit buffer and % starting buffer which eluted the column during the 16-hour run, was predetermined and shown in Fig. 1. These buffers were fed to the column from a gradient mixer - Gilson Ltd. mixograd.

Zinc was measured in the eluates by atomic absorption spectrometry described previously on page 46. The protein was detected by measuring absorbance at 280 nm using an LKB Uvichord II which received the eluates directly from the column. The absorbance of protein in each fraction collected was recorded directly on an LKB chart recorder.

The eluate fractions which contained the Zinc were concentrated to 5 ml by ultrafiltration using PM-10 membranes in a Diaflo concentration cell (Amicon Corp.). These concentrated fractions were further resolved by gel filtration on a 2.6 x 40 cm column of Sephadex G-100.

Seperation of Protein by Gel Filtration

The zinc-rich fractions separated by Ion-exchange chromatography and concentrated as described on page 51 were further resolved by Gel Filtration. The method was as described in the Sephadex booklet by Pharmacia Ltd. (1966).

A slurry of Sephadex G-100 was poured into the columns and allowed to settle. The packing was completed by washing with several column volumes of 0.01 M Ethylene diamine tetra acetic acid (E.D.T.A.), until the bed volume was constant. The 2.6 x 40 cm column was then equilibriated with 0.1 M.Tris, pH 8.2. 500 ml of buffer was required for elution before the pH of the effluent was constant.

The concentrated sample was then applied to the top of the column. 300 ml of 0.1 M.Tris buffer was used to elute the column at a pressure of 50 cm H_2O. 5 ml fractions were collected. The eluted proteins were detected by measuring absorbance at 280 nM with an LKB Uvichord II flow cell which received the eluates directly from the column. The absorbance of protein in each 5 ml fraction collected from the column was recorded directly on an LKB chart recorder.

Molecular Weight Determination of Plasma Proteins

The protein fractions in which Zinc was detected were separated by the previously described methods of Ion-Exchange and Gel Filtration; their molecular weights were then determined by following a similar method to Andrews (1964). A 2.6 x 40 cm column of Sephadex G-100 was packed and eluted by 0.1 M Tris buffer, pH 8.2 as described earlier.

Proteins (Boehringer Ltd.) of known molecular weight shown below, were applied to the column.

Protein	Molecular Weight
Cytochrome C	12,400
Trypsin inhibitor	21,000
Chymotrypsinogen A	25,700
Myoglobin	17,800
Albumin - from bovine serum	67,000
Aldolase	158,000
Catalase	240,000
Thyroglobulin	670,000

The elution volume (Ve) for each protein was determined; the elution volume of Blue Dextran (molecular weight 2×10^6) was determined for the void volume (Vo) of the column. The total volume of the gel bed (Vt) was also determined. The partition coefficient (Kav) was calculated from the following formula:

$$Kav = \frac{Ve - Vo}{Vt - Vo}$$

Calibration curves on logarithmic paper, plotting Kav values against the known molecular weights was carried out according to the method outlined in Sephadex booklet by

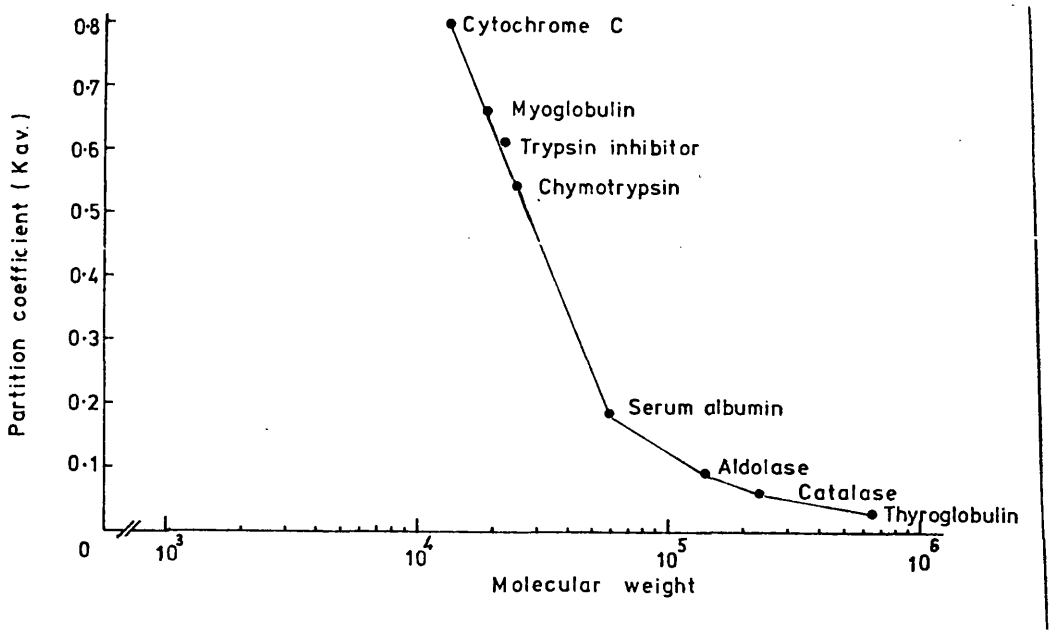


FIG. 2 RELATIONSHIP BETWEEN Kav AND MOLECULAR WEIGHT

Proteins of known molecular weight plotted on a logarithmic scale against their partition coefficients (Kav). Proteins were separated by Gel filtration using sephadex G-100, in 2.6 x 40 cm. columns which were equilibrated with 0.1M-tris hydrochloride buffer pH 8.2.

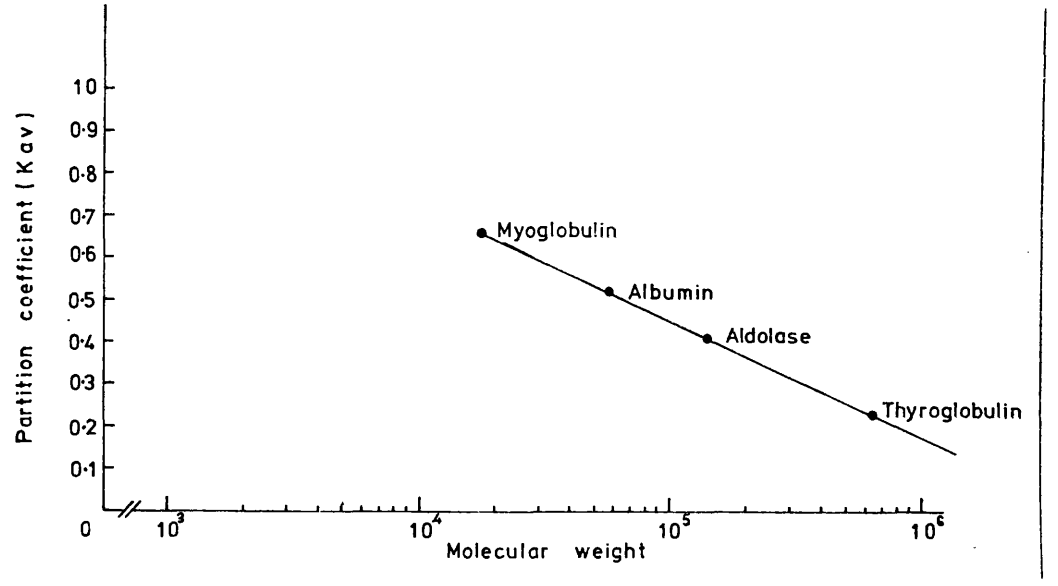


FIG. 3 RELATIONSHIP BETWEEN Kav AND MOLECULAR WEIGHT

Proteins of known molecular weight are plotted on logarithmic scale against their partition coefficients (Kav).
Proteins were seperated by Gel filtration using Ultrogel AcA 22, in 2.6 x 40 cm. columns which were equilibriated with 0.1M-tris hydrochloride buffer pH 8.2.

Pharmacia Ltd. These are shown in figures 2 and 3.

The zinc-rich protein fractions were applied to the column; their corresponding elution volumes were determined and their Kav values calculated. Their molecular weights were then read from the calibration curve.

DETERMINATION OF PROTEIN IN SOLUTION

The procedure was similar to the one proposed by Lowry, Roseburgh, Farr (1951). A modification was made by shortening the time from 30 to 20 minutes for primary reaction product to be converted to the blue end product. This was done by immersing the test tubes in water at 35°C as recommended by Reider (1959).

Reagents:-

A: 2% Na_2CO_3 in 0.1 M.NaOH

B: 0.5% $CuSO_4.5H_2O$ in 1% sodium tartrate

C: 1 ml of reagent B mixed with 50 ml of reagent A, this solution was stable for 1 day.

D: Commercially available Folin-Ciocalteu reagent was diluted to 1M in acid.

Method

0.2 ml plasma was added to 1 ml of reagent C. after mixing, the solution was left for 10 minutes at room temperature. 0.1 ml of reagent D was added and mixed rapidly with the solution because reagent D was unstable.

The primary reaction product was slowly converted to the blue end product and the reaction mixture was allowed to stand in water at 35°C for 20 minutes. This gave a constant spectrophotometric reading.

The solutions were read spectrophotometrically at 750 nm and the concentrations determined from a calibration curve, which was reasonably consistent.

CELLULOSE ACETATE ELECTROPHORESIS OF PLASMA PROTEINS

This method is similar to the standard method of Kohn (1958). Plasma proteins were separated by electrophoresis on cellulose acetate membranes using Tris-barbital buffer, pH 8.8. The electrophoretograms were stained with Ponceau S and quantified by transmission densitometry using a Joyce Loeble Chromoscan 200.

The reagents and procedure used were the same as described by Henry et al. (1974).

The cleared cellulose acetate membranes were scanned by an integrating densitometer using a 570 mm filter and a 0.1 x 6 mm slit. The densitometer scan was adjusted to give a chart reading of 80% transmission with albumin. This was to make certain that the densitometer readings were linearly related to concentration of plasma protein fractions.

The densitometer recorded the integration counts for each protein fraction. The concentration of protein in each fraction in g/100 ml was calculated as follows:-

$$\frac{\text{Integration counts for each fraction}}{\text{Total integration counts}} \times \begin{array}{l}\text{concentration of}\\ \text{total plasma protein}\\ \text{(g/100 ml)}\end{array}$$

The total plasma protein concentration was determined by the method described on page 56.

POLYACRYLAMIDE DISC GEL ELECTROPHORESIS

This technique was used for locating the α2-macroglobulin protein in fractions separated by ion-exchange and gel filtration chromatography.

The method is very similar to the one described by Ornstein and Davies (1964). Its high resolving power depends on two physical effects:-

1. The molecular sieving effect by which the individual molecules are electrophoretically seperated on the basis of their molecular weight and tertiary structure.
2. The electrocharge effect by which the sample molecules are fractionated according to their nett electric charge.

Preparation of the Gel and Buffer

Solution	Content
1	30.0 g of acrylamide monomer (Eastman 5521) and 1 g of N,N'-methylene-bis-acrylamide (Eastman 8383) - Bis - in 123 ml deionised water. A permutit mixed bed ion-exchanger was used to obtain deionised water.
2	1.3 ml of N_4 tetramethylethylenediamine (Eastman 8178) - TEMED - added to 500 ml deionised water to make 0.28% vol/vol.
3	0.7 g ammonium persulphate dissolved in deionised water and made up to 500 ml. The final solution was 0.14% wt/vol.
4	29.0 glycine and 6.0 g Tris (hydroxymethyl aminomethane) dissolved in 980 ml deionised water.

Solution	Content
5	Buffer solution - 29.0 g of glycine and 6.0 g Tris were dissolved in deionised water to which 5.0 ml M.HCl was added. The mixture was titrated to pH 8.1 and made up to 980 ml with deionised water. A dilution of 1:10 of this buffer was used in the electrode compartments of the apparatus.

All these solutions were thoroughly mixed and filtered before they were stored in dark bottles and kept in a refrigerator.

The gels were prepared by mixing:

(a) 2 volumes of solutions 1 (acrylamide and bis)

(b) 1 volume of solution 2 (TEMED)

(c) 4 volumes of solution 3 (ammonium persulphate)

(d) 1 volume of solution 4 (glycine/tris mixture)

40 ml of this mixture was sufficient for eight gels. This gel mixture was poured into tubes 7.5 cm long which were siliconised with dichloro-dimethylsilane. The siliconisation facilitated extracting the gels from the tubes for staining. The surface of each gel mixture (before polymerisation) in the tubes was covered with water soon after pouring. Polymerisation of the gel was complete after 30 minutes. The water on the top of each gel was shaken off and eight tubes then placed in a disc electrophoresis apparatus (Shandon Scientific Co. Ltd.). Buffer was poured into each electrode compartment. 0.1 ml of the sample containing α_2-macroglobulin was then dispersed on to the top of each gel. The cathode and anode electrodes were then connected to upper and lower buffer reservoirs

respectively. The power pack (Gallenkamp Ltd.) was adjusted to give a constant current of 1-2 ma per tube for three minutes and then increased to give 4 ma per tube for thirty minutes.

The gels were then taken out of the tubes for staining with coomassie blue. Firstly, the gels were suspended in 12.5% TCA for 30 minutes, followed by immersion in a freshly prepared 1:20 mixture of a 1% aqueous stock solution of coomassie blue and 12.5% TCA for 30 minutes.

The gels were then transferred to 10% TCA for preservation and scanning.

Determination of Plasma Alkaline Phosphatase Activity

The alkaline phosphatase activity in lamb plasma was determined by following the standard technique in the Sigma Technical Bulletin No. 104. All reagents were obtained from the Sigma-London Chemical Co. Ltd.

The principle of the method depends on the substrate p-nitrophenyl phosphate which is colourless in acid and alkali solution, but after hydrolysis by alkaline phosphatase, phosphate and p-nitrophenol are produced thus:-

$$\underset{\text{phosphate}}{\text{p-nitrophenol}} + H_2O \xrightarrow{\text{phosphatase}} \underset{\text{(yellow in alkali)}}{\underset{\text{colourless in acid}}{\text{p-nitrophenol}}} + H_3PO_4$$

The plasma was incubated with the buffered substrate and the reaction stopped by sodium hydroxide. This stopped further enzyme activity and developed the yellow colouration which was stable for several hours. The amount of p-nitrophenol liberated after a certain time was proportioned to the phosphatase activity and the optical density was measured at 410 nm in a Unicam 500 spectrophotometer.

The alkaline phosphatase units were determined from a calibration curve obtained with standard solutions; these solutions were made up from the working standard mentioned below.

The units of enzyme activity obtained were based on the Sigma units/ml of plasma. One Sigma unit of phosphatase liberated 1 micromole of p-nitrophenol per hour under specified conditions (1 micromole = 0.1391 mg).

Preparation of Calibration Curve

The working standard

0.02 N sodium hydroxide solution was added to 0.5 ml p-nitrophenol standard solution Sigma stock No. 104-1 (10 millimoles per litre) and made up to 100 ml.

The standard solutions were made as tabulated in the following chart in which the recommended equivalent Sigma units of enzyme/ml plasma are also given with each standard.

Tube No.	Vol. of working standard (ml)	Vol. of 0.02N NaOH solution (ml)	Optical density	Equivalent to the following Sigma units/ml plasma alkaline phosphatase
1	1.0	10.0	Readings obtained at 410 nm using 0.02M NaOH as blank for each mixture.	1.0
2	2.0	9.0		2.0
3	4.0	7.0		4.0
4	6.0	5.0		6.0
5	8.0	3.0		8.0
6	10.0	1.0		10.0

The spectrophotometer was set at zero optical density, using 0.02M. NaOH solution as a reagent blank in 1 cm cuvette at 410 nm.

The optical density was recorded using the standard solution in the six tubes prepared as tabulated in the chart above.

The optical density thus obtained for each tube was plotted against the corresponding recommended alkaline phosphatase Sigma units and a graph showing the standard curve was therefore prepared.

The working standard and the standard solutions were freshly prepared at the time of enzyme assay and were discarded after use.

DETERMINATION OF PLASMA BOUND ZINC BY ULTRAFILTRATION

Ultrafiltration was carried out in a Diaflo cell made by Amicon Ltd., High Wycombe, Bucks. A filter UM.05, in this cell under a pressure of 55 p.s.i., retained proteins with molecular weights greater than 500.

Pilot recovery experiments showed that this method was 98 per cent efficient in retaining proteins.

Blood plasma was diluted 1:3 with deionized water before ultrafiltration. These procedures were carried out at $4^{o}C$.

The Zinc concentration in the retained proteins was determined by Atomic absorption spectroscopy as described previously.

CHAPTER 5

Investigation of Dietary Zinc Deficiency on the Performance of Growing Lambs

Introduction

The objective of this investigation was to give young lambs a diet which had a Zinc content lower than had previously been reported in the literature and to record the consequence of consuming such a diet on performance. These results would decide whether a homeostatic control mechanism for Zinc can function in severe dietary Zinc deficiency (0.9 mgZn./Kg) as suggested by Miller (1969) and Miller et al (1972).

Performance was assessed by measuring food intake, growth rate and food conversion efficiency.

It was anticipated from previous work (Mills et al 1967) that Zinc deficiency would cause poor performance. It was, therefore, the intention to see whether good performance could be restored to Zinc deficient lambs by giving a Zinc supplemented diet (40 mgZn./kg diet) and the time needed if this was achieved.

The effect of dietary Zinc deficiency on performance of male and female lambs was recorded throughout.

Experimental Design

15 male and 15 female lambs were arranged in three treatment groups and housed as described earlier in "General Experimental Design and Treatment". Each treatment group contained 5 lambs.

The experiment was twelve weeks in duration and divided into two periods of six weeks each.

RESULTS

The three criteria of performance-food intake, growth rate, and food conversion are presented seperately.

The analysis of variance tables are shown in Appendices 1-4.

The mean Zinc intake for each group during each period is shown in Tables 1a and 1b. The Zinc intake was calculated from the amount of food intake and a mean 0.9 mg Zn./Kg diet.

The amount of Zinc intake per week for the Zinc deficient group was reasonably constant.

The amount of Zinc intake per week in Period 1 for the Ad lib control group was slightly higher than for the Pair fed group, but the difference was not significant.

TABLES 1a AND 1b

The Mean Zinc Intake (mg) per Week for Different Treatments in Periods 1 and 2

(Zinc deficient basal diet contained 0.9 mg Zn./Kg; Zinc supplemented basal diet contained 40.0 mg Zn./Kg.)

Table 1a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
3	5.3	232.9	268.3
4	5.4	239.5	273.8
5	5.5	245.7	278.9
6	5.6	248.7	276.8

Standard Error ± 3.13

Means calculated from 10 values

Table 1b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
9	5.3	235.2	233.6
10	5.4	241.0	237.4
11	5.4	241.2	238.8
12	5.5	245.7	241.1

Standard Error ± 1.97

Means calculated from 10 values

FOOD INTAKE

Period 1

Table 2a and Fig. 4 show clearly that lambs fed Zinc deficient diets were consuming less food than controls throughout this period. The mean food intake per week of the Zinc deficient groups - 6042.75 g - was significantly ($P<0.001$) lower than the mean food intake - 6861.5 g - for the ad lib control groups during the last four weeks of the period.

Figure 4 shows that the food intake of the zinc deficient groups decreased rapidly during the first week of the experiment. Although there was a slight increase in mean food intake of these groups during the last four weeks of the period, it did not recover either to initial or control values.

The Effect of Zinc Deficiency on Food Intake in Male and Female Lambs

The effect was similar in both sexes as shown in Tables 3a and 3b. In both the zinc deficient males and females the mean food intakes of 5727.5 g and 6358.0 g were significantly ($P<0.001$) less than mean food intakes of their controls, being 6435.0 g and 7288.0 g respectively.

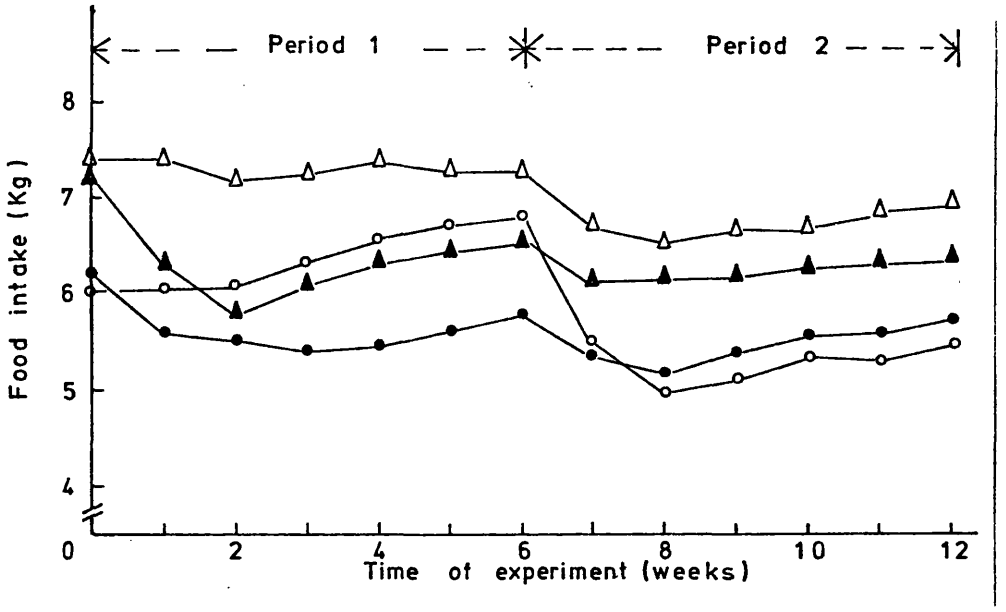


FIG.4 MEAN FOOD INTAKE OF LAMBS

Zinc deficient males in Period 1 and Zinc supplemented ad lib in Period 2

Ad lib male controls in Period 1 and Zinc deficient in Period 2.

Zinc deficient females in Period 1 and Zinc supplemented ad lib in Period 2.

Ad lib female controls in Period 1 and Zinc deficient in Period 2.

Period 2

Figure 4 and Tables 2a and 2b show that feeding Zinc deficient diets to lambs in this period decreased the food intake in a similar way to the results found in Period 1.

During the first two weeks (weeks 7 and 8) in Period 2 the food intake of the zinc deficient groups decreased significantly ($P<0.001$) from a mean 6,925.0 g in week 6 to a mean intake of 5,850.0 g in week 8.

In the remaining four weeks of Period 2 the mean food intake of the zinc deficient groups shown in Figure 4 and Table 2b increased significantly ($P<0.001$) from 5881.0 g in week to 6142.0 g in week 12. This was similar to the significant ($P<0.001$) increase in food intake for the ad libitum control group from a mean 5840.0 g in week 9 to 6029.0 g in week 12 during the same four weeks. This showed that the initially lowered food intake response to Zinc deficiency was overcome after two weeks.

Effect of Zinc Supplementation

The mean food intake of the ad libitum control group (previously Zinc deficient in Period 1) which was 6219.0 g at the beginning of this period decreased during the first weeks of consuming the zinc supplemented diet. During the remaining weeks of this period the food intake increased until it reached a mean of 6029.0 g in week 12. It must be noted, however, that this mean value for food intake in the ad libitum control group was significantly lower ($P<0.01$) than mean food intake for ad libitum control group in Period 1 after a similar time.

TABLES 2a AND 2b

Mean food intakes (g/week) of lambs on different dietary treatments in Periods 1 and 2

Table 2a Period 1

Weeks of experimental period	Treatments	
	Zinc deficient group	Ad libitum control group
3	5822.0	6708.0
4	5988.0	6846.0
5	6142.0	6973.0
6	6219.0	6919.0

Standard Error ± 23.2

Means calculated from 10 values

Table 2b Period 2

Weeks of experimental period	Treatments	
	Zinc deficient group	Ad libitum control group
9	5881.0	5840.0
10	6026.0	5935.0
11	6028.0	5971.0
12	6142.0	6029.0

Standard Error ± 12.45

Means calculated from 10 values

TABLES 3a AND 3b

The Effect of Zinc Deficiency on Food Intake (g/week) in Male and Female Lambs

Table 3a Period 1

Sex	Treatments	
	Zinc deficient group	Ad libitum control group
Male	5727.5	6435.0
Female	6358.0	7288.0

Standard Error ± 16.43

Means calculated from 5 values

Table 3b Period 2

Sex	Treatments	
	Zinc deficient group	Ad libitum control group
Male	5355.0	5585.0
Female	6683.5	6302.5

Standard Error ± 8.80

Means calculated from 5 values

Effect of Zinc Deficiency on Food Intake in Male and Female Lambs

Comparison of mean food intakes shown in Figure 4 and Tables 3a and 3b of male and female lambs on Zinc deficient and Zinc supplemented diets showed that there was little difference between the male and female intakes of Zinc deficient diet when the difference in intake of the supplemented diet by the control groups was taken into account.

Growth Rate

A comparison of the changes in live weight of lambs on Zinc deficient diets with lambs on Zinc supplemented diets for two periods of six weeks is shown in both Figure 5 and Figure 6. The mean growth rates of these lambs during the last four weeks of each period are shown in Table 4a and 4b.

Period 1 The consumption of Zinc deficient diets caused slower growth rates than in either of the supplemented control groups of lambs. There was little difference in growth rates between the groups for the first three weeks, but during the fourth week the mean growth rate of the Zinc deficient lambs, 280.0 g/week, was significantly lower ($P<0.001$) than mean growth rates of lambs in both the ad lib control (1118.0 g/week) and the pair fed control (696.0g/week) groups. This significantly lower growth rate persisted until the end of the period.

There was a tendency for mean growth rate of the Zinc deficient group to continue decreasing during the last three weeks of the period from 280.0 g/week in Week 3, to 235 g/week in Week 6. This suggested that lambs had not adapted fully to low Zinc diets.

Although the mean growth rate of female lambs fed Zinc deficient diets (Table 5a) was lower than the male lambs, the difference was not significant. Thus the growth rate of females was not uniquely affected by Zinc deficiency compared with the males.

Period 2 In this period the Zinc deficient group showed no significant difference in growth rates from the Zinc deficiency group in Period 1; the mean growth rates for Zinc deficient

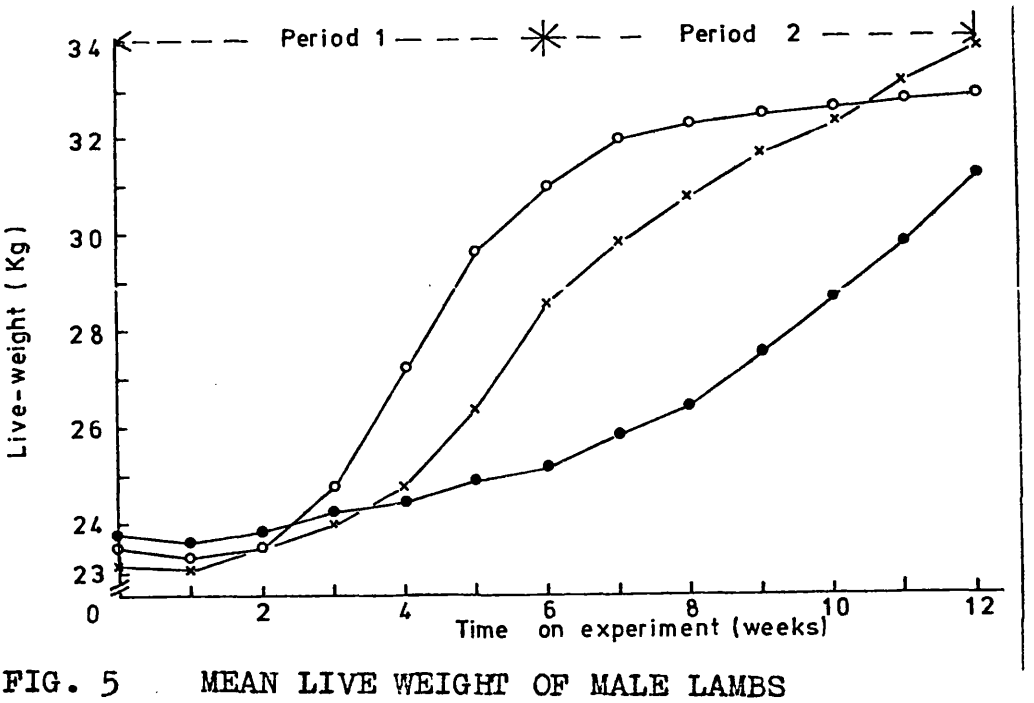


FIG. 5 MEAN LIVE WEIGHT OF MALE LAMBS

●—●—● Zinc deficient in Period 1, Zinc supplemented in Period 2.

o—o—o Ad lib controls in Period 1, Zinc deficient in Period 2.

×—×—× Pair fed control in Periods 1 and 2.

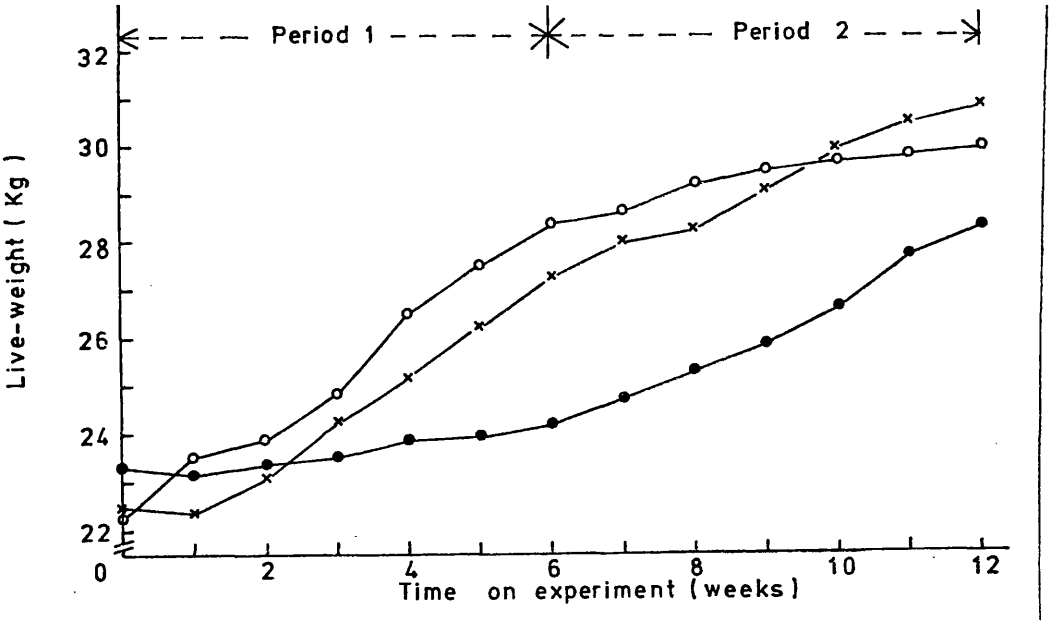


FIG. 6 MEAN LIVE WEIGHT OF FEMALE LAMBS

●—●—● Zinc deficient in Period 1, Zinc supplemented in Period 2

o—o—o Ad lib control in Period 1, Zinc deficient in Period 2.

×—×—× Pair fed control in Periods 1 and 2.

TABLES 4a AND 4b

Mean Growth Rate (g/week) on Different Dietary Treatments in Periods 1 and 2

Table 4a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
3	280.0	696.0	1118.0
4	268.0	840.0	1975.0
5	232.0	1230.0	1700.0
6	235.0	1715.0	1103.0

Standard Error ± 4.40

Means from 10 values

Table 4b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
9	290.0	835.0	771.0
10	133.0	715.0	900.0
11	224.0	738.0	1007.0
12	183.0	702.0	1005.0

Standard Error ± 86.9

Means from 10 values

TABLES 5a AND 5b

The Effect of Zinc Deficiency on Growth Rate (g/week) in Male and Female Lambs

Table 5a Period 1

Sex	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
Male	290.0	1250.0	1825.0
Female	217.5	990.0	1123.0

Standard Error ± 3.11

Means from 5 values

Table 5b Period 2

Sex	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
Male	200.0	792.0	1205.0
Female	215.0	702.5	636.5

Standard Error ± 61.5

Means from 5 values

groups in both periods were 254 g/week in Period 1 and 207 g/week in Period 2.

The mean growth rates of the Zinc deficient group in Period 2 continued to decrease significantly ($P<0.01$) during the last three weeks from 290.0 g/week in Week 9, down to 183.0 g/week in Week 12.

The mean growth rates of the pair-fed control group shown in Table 4b also tended to decrease, but the difference between the means (835.0 g/week in Week 9 and 702.0 g/week in Week 12) was not significant.

Effect of Zinc Supplementation

It is interesting to note in Table 4b the rapid increase in growth rates for the ad lib control group. This group, previously the Zinc deficient group in Period 1. responded to supplemented Zinc by growth rates increasing significantly ($P<0.001$) from 771.0 g/week in Week 3 to 1005.0 g/week in Week 6.

The results of feeding a Zinc deficient diet in this period appears from Table 5b to have had more effect on the mean growth rate of male than female lambs. The mean growth rate of males was significantly ($P<0.001$) lower than the mean growth rate of females. A comparison of Zinc deficient male and female line weights in Figures 5 and 6 respectively, suggests however, that there was little difference between the males and females after consuming the Zinc deficient diet for three to four weeks.

Food Conversion Efficiency (F.C.E.)

$$\text{F.C.E.} = \frac{\text{Mean food intake per week}}{\text{Mean growth rate per week}}$$

(N.B. The higher values of F.C.E. are poorer converters of food)

Tables 6a and 6b show that Zinc deficient groups of lambs had higher F.C.E. values than control groups in both Periods 1 and 2. The Zinc deficient lambs were, therefore, less efficient at converting food.

In Period 1 the mean F.C.E. for the Zinc deficient group (27.31) was significantly higher ($P < 0.001$) than either the pair fed group (8.70) or the ad lib control group (7.56).

It is noticeable that the ad lib control group in Period 2 (Zinc deficient in Period 1) responded to the supplemented Zinc diet in Period 2 by low F.C.E. values. The lambs were, therefore, better converters of food than when they consumed a Zinc deficient diet.

In both periods 1 and 2, a comparison of mean F.C.E. for male and female lambs (Tables 7a and 7b respectively) fed Zinc deficient diets suggests that the females were poorer converters of the Zinc deficient diet, than males. These differences between male and female F.C.E. however, were not statistically significant.

TABLES 6a AND 6b

Mean Food Conversion Efficiency (F.C.E.)
for Different Dietary Treatments in Periods 1 and 2

Table 6a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
3	18.9	10.3	6.3
4	26.6	7.4	3.8
5	22.5	5.3	5.2

Standard Error ± 2.5

Means from 10 values

Table 6b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
9	12.5	7.9	9.0
10	46.7	9.2	7.6
11	18.8	8.8	6.5
12	31.2	9.0	7.3

Standard Error ± 12.4

Means from 10 values

TABLES 7a and 7b

The Effect of Zinc Deficiency on F.C.E. in Male and Female Lambs

Table 7a Period 1

Sex	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
Male	18.4	7.2	4.1
Female	28.2	6.5	6.9

Standard Error ± 1.8

Means from 5 values

Table 7b Period 2

Sex	Treatments		
	Zinc deficient group	Pair fed control group	Ad libitum control group
Male		7.4	4.9
Female	51.1	10.0	10.2

Standard Error ± 8.8

Means from 5 values

CHAPTER 6

A Study of Clinical Observations and Plasma Alkaline Phosphatase Activity

Introduction

Plasma alkaline phosphatase activity was measured for two reasons. Firstly, Day and McCallom (1940), Luecke et al. (1956) and Miller et al. (1965) all found that a reduction in plasma activity of this enzyme during Zinc deficiency in cows and pigs correlated closely with the severity of clinical lesions. Secondly, the enzyme activity was thought to be a likely indicator of the physiologically available plasma Zinc, and therefore a further parameter in assessing the presence of a homeostatic mechanism for Zinc in plasma.

Photographs were taken at weekly intervals to record the external appearance of Zinc deficient lambs during the six week period, in comparison with Zinc supplemented lambs.

Plasma Alkaline Phosphatase Activity

The results for mean alkaline phosphatase activity are shown in Figure 7 and Tables 8a and 8b.

Period 1

The mean activity values for this enzyme decreased in lambs fed zinc deficient diets. The mean activity decreased significantly ($P < 0.01$) during the six weeks, from 9.10 sigma units/ml at the beginning of the period to 2.04 sigma units/ml in week 6.

Further, the mean alkaline phosphatase activity of the zinc deficient group 3.38 sigma units/ml was significantly lower ($P < 0.01$) than the mean 8.77 sigma units/ml for the pair-fed control group.

Effect of Zinc Deficiency on Alkaline Phosphatase in Males and Females

The results are shown in Table 9a. There was a significantly lower ($P < 0.01$) mean alkaline phosphatase activity 1.96 sigma units/ml in zinc deficient females than in males 4.79 sigma units/ml fed a similar diet. There was no significant difference between means in pair-fed control male and female lambs.

Period 2

The results in Table 8b showing the effect of zinc deficiency on alkaline phosphatase activity were similar to results in Period 1. The mean activity of the zinc deficient group decreased significantly ($P < 0.01$) from 8.93 to 1.09 sigma units/ml over the six week period.

The mean alkaline phosphatase activity (1.80 sigma units/ml) was significantly lower ($P < 0.01$) than 8.68 sigma units/ml for the pair-fed control group.

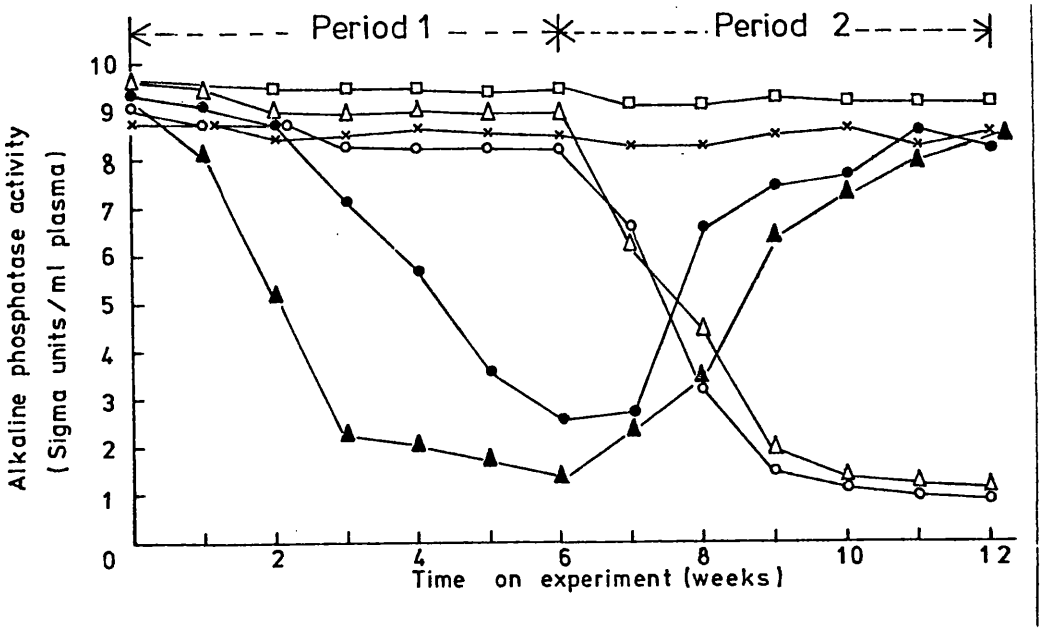


FIG. 7 MEAN ALKALINE PHOSPHATASE ACTIVITY IN LAMB PLASMA

●—●—● Zinc deficient males in Period 1, Zinc supplemented Ad lib in Period 2.

○—○—○ Ad lib male control in Period 1, Zinc deficient in Period 2.

×—×—× Pair fed male control in Periods 1 and 2.

▲—▲—▲ Zinc deficient females in Period 1, Zinc supplemented Ad lib in Period 2.

△—△—△ Ad lib female control in Period 1, Zinc deficient in Period 2.

□—□—□ Pair fed female control in Periods 1 and 2.

Effect of Zinc Deficiency on Alkaline Phosphatase in Males and Females

In contrast to the results in Period 1, there was a significantly lower ($P < 0.05$) mean activity 1.19 sigma units/ml in zinc deficient males than in females 2.41 sigma units/ml fed a similar diet. No significant difference was detected between means in pair-fed control male and female lambs.

Effect of Zinc Supplementation

The mean alkaline phosphatase activity 2.04 sigma units/ml in the ad lib control group (previously zinc deficient) at the commencement of Period 2, increased significantly ($P < 0.01$) after six weeks, to 8.27 sigma units/ml. The activity of this enzyme in the pair-fed control group at week 6 was 8.64 sigma units/ml.

TABLES 8a AND 8b

Mean weekly alkaline phosphatase activity (sigma units) in plasma of lambs on different dietary treatments in Periods 1 and 2

Table 8a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	4.79	8.71	8.87
4	3.95	8.68	8.89
5	2.73	8.66	8.71
6	2.04	8.93	8.65

Standard error ± 0.25

Means calculated from 10 values

Table 8b Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	2.23	6.61	8.72
10	2.73	7.19	8.68
11	1.15	8.51	8.68
12	1.09	8.27	8.64

Standard error ± 0.46

Means calculated from 10 values

TABLES 9a and 9b

The effect of zinc deficiency on plasma alkaline phosphatase in male and female lambs

Table 9a Period 1

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	4.79	8.43	8.39
Female	1.96	9.06	9.16

Standard error ± 0.35

Means calculated from 5 values

Table 9b Period 2

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	1.19	7.95	8.46
Female	2.41	7.33	8.90

Standard error ± 0.33

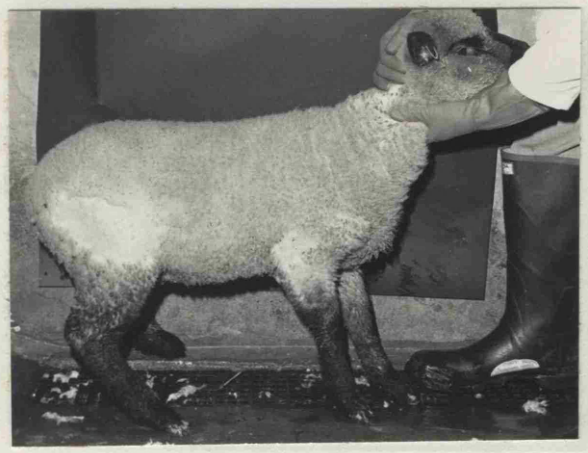
Means calculated from 5 values

Units of enzyme activity based on sigma units/ml plasma. One sigma unit of phosphatase liberated 1 micromole of p-nitrophenol per hour (1 micromole = 0.1391 mg.)

Clinical Observations in Zinc Deficient Lambs

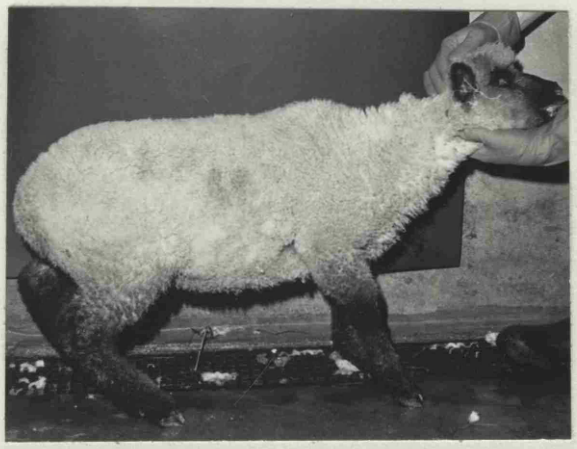
Appearance After Consuming The Zinc Deficient Diet For One Week

Zinc Deficient Lamb



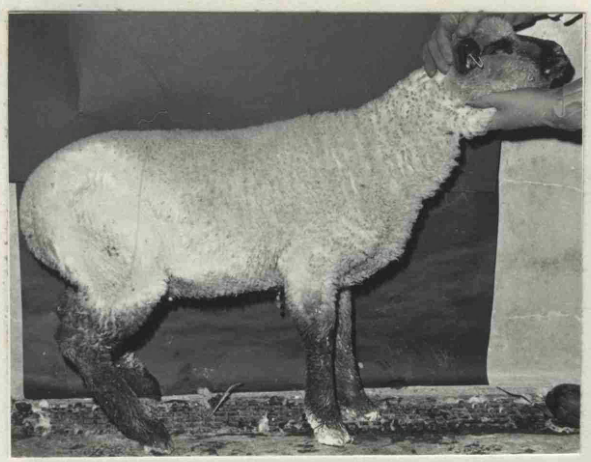
The only symptom was loss of wool from the top of legs as shown.

Zinc Supplemented Lamb



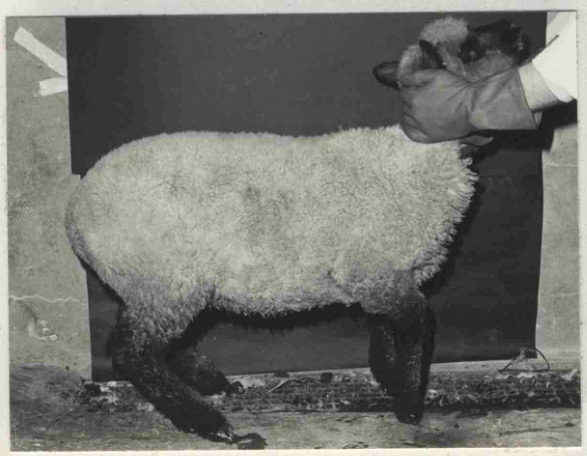
Appearance After Consuming The Zinc Deficient Diet for Two Weeks

Zinc Deficient Lamb



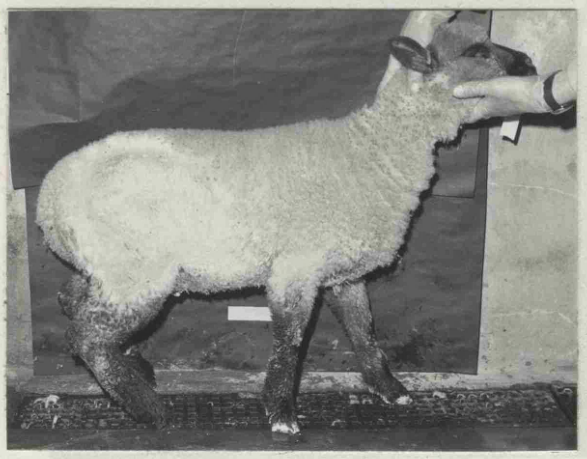
Wool was lost from a larger area; also there was obvious loss of wool from the face.

Zinc Supplemented Lamb



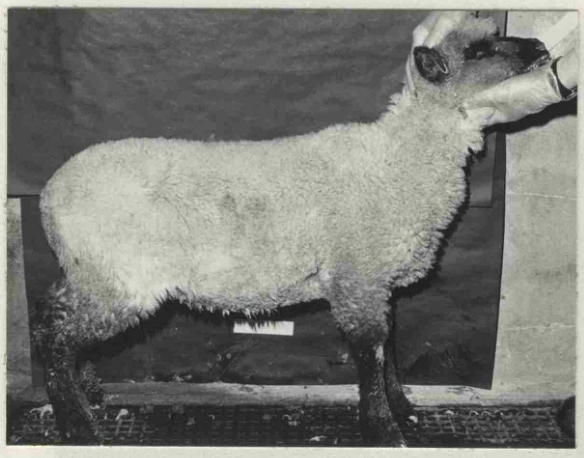
Appearance After Consuming The Zinc Deficient Diet for Three Weeks

Zinc Deficient Lamb



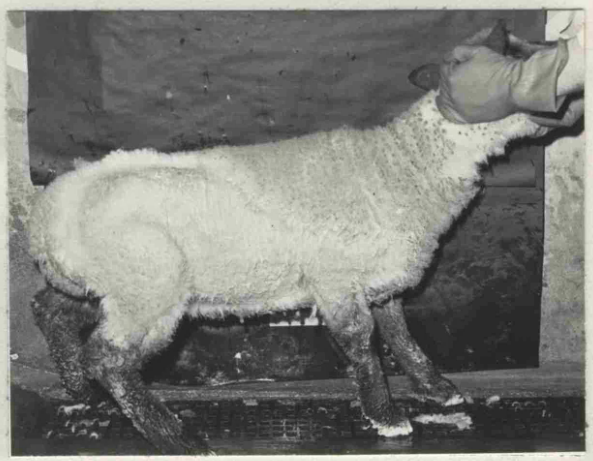
The loss of wool continued, leaving a clear view of large areas of skin. Soft spongy outgrowths were observed on the feet which periodically haemorrhaged. Pinprick sized haemorrhages were occurring on the surface of skin.

Zinc Supplemented Lamb



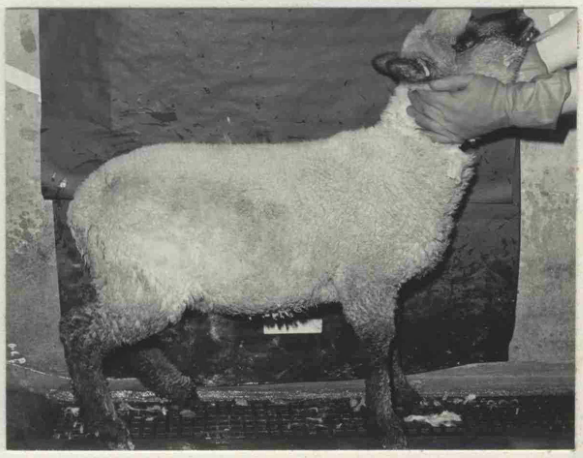
Appearance After Consuming The Zinc Deficient Diet For Four Weeks

Zinc Deficient Lamb



The previously mentioned symptoms continued; also the wool was very thin, brittle and easily plucked from the lamb's coat.

Zinc Supplemented Lamb



Appearance After Consuming The Zinc Deficient Diet For Five Weeks

Zinc Deficient Lamb

In addition to the previous symptoms, excessive salivation was observed in all lambs. This is in contrast to observations by Mills et al. (1967) who observed this symptom by the 10-12th day of Zinc deficiency.

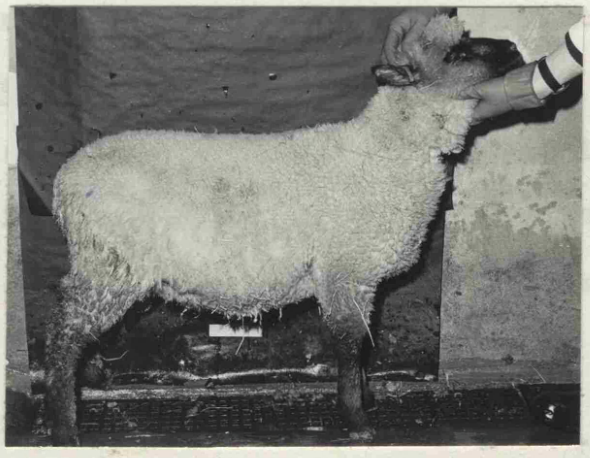
Zinc Supplemented Lamb

Appearance After Consuming The Zinc Deficient Diet For Six Weeks

Zinc Deficient Lamb

Similar symptoms were observed as during the fifth week of Zinc deficiency.

Zinc Supplemented Lamb



CHAPTER 7

The Effect of Dietary Zinc Deficiency on Plasma Zinc and Protein Levels

Introduction

Dietary Zinc is usually absorbed from the digestive tract and subsequently enters the blood where it is transported to the different parts of the body.

In the previous experiment the performance of lambs decreased as a result of low dietary Zinc; it would be expected that plasma Zinc would have shown a similar decrease.

Most of the plasma Zinc is bound to protein in human plasma according to Prasad and Oberleas (1970). On the assumption that a similar binding of Zinc occurs in lambs, then Zinc deficiency would pose the questions:-

(a) Does the concentration of plasma proteins change in Zinc deficient lambs?

(b) Is there any suggestion that a homeostatic mechanism exists in lambs which maintains plasma Zinc and/or plasma proteins during Zinc deficiency.

Experimental Design

The experiment was set up as previously described for investigating the effect of Zinc deficiency on lamb performance. Determination of the following were carried out:-

plasma zinc, total plasma protein, albumin, globulins, and individual globulin fractions i.e. $\alpha 1$-, $\alpha 2$-, β-, and g-globulins.

Results

Analysis of variance tables are shown in Appendices 5-13.

The mean Zinc intake for each group of lambs during each period was shown in Tables 1a and 1b.

In the presentation of the following results the Zinc deficient group means are compared with the pair-fed control group means. The results for the ad lib control group given in each table are similar on each occasion to the results for the pair-fed control group.

Plasma Zinc Concentration

Figures 8 and 9 and Tables 10a and 10b show that the plasma zinc concentration in the zinc deficient groups was lower than in the control groups during Periods 1 and 2.

Period 1

The mean plasma zinc concentration in the zinc deficient groups decreased significantly ($P < 0.001$) from an initial 1.15 µg/ml to 0.27 µg/ml after six weeks. This low plasma zinc concentration was significantly lower ($P < 0.001$) than the pair-fed control mean of 1.14 µg/ml.

Most of the decrease in the plasma zinc concentration occurred during the first week of consuming the zinc deficient diet; the mean plasma zinc decreased significantly ($P < 0.001$) from 1.15 ug/ml to 0.37 µg/ml.

Effect of zinc deficiency on plasma zinc in males and females

An analysis of variance showed no significant difference between plasma zinc concentration in male and female lambs fed on zinc deficient diets. The means are shown in Table 11a.

Period 2

The mean plasma zinc concentration in the zinc deficient groups decreased significantly ($P < 0.001$) from a mean 1.15 µg/ml to 0.18 µg/ml after six weeks. During this time the mean 0.18 µg/ml for zinc deficient groups was significantly lower ($P < 0.001$) than the mean for pair-fed groups, 1.13 µg/ml.

As in Period 1 most of the decrease in plasma zinc concentration occurred in the first week of consuming zinc deficient diets; during this time the zinc concentration decreased significantly ($P < 0.001$) from a mean of

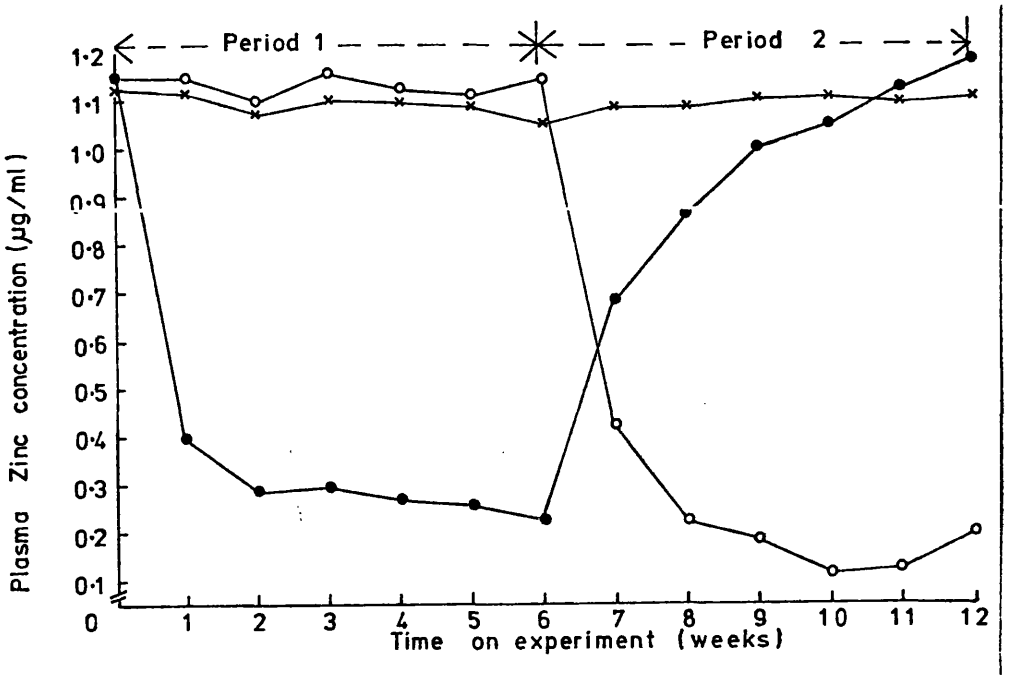


FIG. 8 MEAN PLASMA ZINC CONCENTRATION IN MALE LAMBS

●—●—● Zinc deficient in Period 1, Zinc supplemented in Period 2.

o—o—o Ad lib control in Period 1, Zinc deficient in Period 2.

×—×—× Pair fed control in Periods 1 and 2.

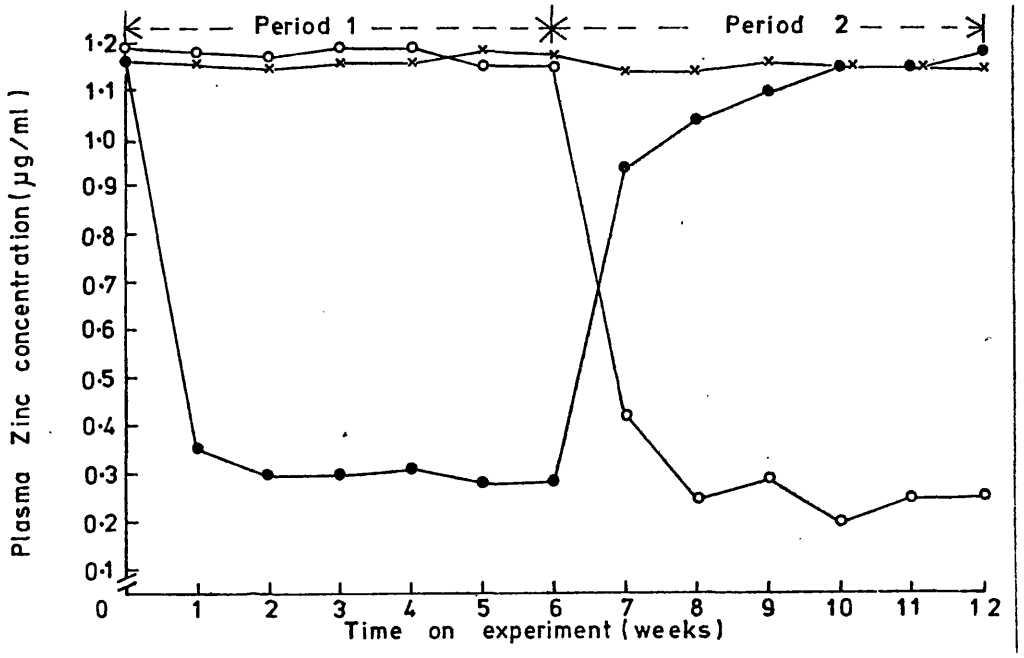


FIG. 9 MEAN PLASMA ZINC CONCENTRATION IN FEMALE LAMBS

●—●—● Zinc deficient in Period 1, Zinc deficient in Period 2.

o—o—o Ad lib control in Period 1, Zinc deficient in Period 2.

×—×—× Pair fed control in Periods 1 and 2.

TABLES 10a AND 10b

Mean plasma zinc concentrations (µg/ml) of lambs on different dietary treatments in Periods 1 and 2

Table 10a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	0.301	1.172	1.139
4	0.293	1.160	1.134
5	0.274	1.134	1.144
6	0.248	1.154	1.136

Standard error ± 0.013

Means from 10 values

Table 10b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	0.236	1.041	1.130
10	0.096	1.099	1.135
11	0.208	1.145	1.131
12	0.169	1.170	1.127

Standard error ± 0.025

Means from 10 values

TABLES 11a AND 11b

The effect of zinc deficiency on plasma zinc in male and female lambs

Table 11a Period 1

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	0.265	1.139	1.112
Female	0.293	1.171	1.165

Standard error ± 0.010

Means from 5 values

Table 11b Period 2

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	0.177	1.088	1.108
Female	0.177	1.139	1.154

Standard error ± 0.018

Means from 5 values

1.150 µg/ml to 0.177 µg/ml.

Effect of Zinc Supplementation

The lambs in the ad lib group (previously zinc deficient in Period 1) responded rapidly, as seen in Figures 8 and 9, to the zinc supplemented diet containing 40 mg/kg. However, these lambs had to be fed the zinc supplemented diet for four weeks, before the mean plasma zinc concentration was restored to levels similar to those in the pair-fed control groups. There was no significant difference between the mean plasma zinc concentration - 1.10 µg/ml for the ad lib control, and 1.13 µg/ml for pair-fed control groups.

These results, therefore, showed that diets containing 40 mg/kg zinc could raise plasma zinc concentration from 0.18 µg/ml to 1.1 µg/ml in four weeks.

Effect of Zinc Deficiency on Plasma Zinc in Males and Females

There was no significant difference between plasma zinc concentration in zinc deficient male and female lambs. The means are shown in Table 11b. It is interesting to note that Lindeman et al. (1971) and Halstead et al. (1970) found that plasma zinc concentrations of women were approximately 5 mg/100 ml lower than those of men.

Plasma Proteins

Total Plasma Protein Concentration

The effect of zinc deficient diets on total plasma protein concentration is shown in figures 10 and 11 and Tables 12a and 12b. During both periods, the plasma protein concentration increased in the zinc deficient lambs.

Period 1

The mean plasma protein concentration 6.20 g/ml of the zinc deficient group increased significantly ($P < 0.001$) during six weeks to a mean of 9.43 g/100 ml.

The mean plasma protein concentration 8.46 g/100 ml of the zinc deficient groups for the period was significantly higher ($P < 0.001$) than the mean 6.17 g/100 ml for the pair-fed control group.

Effect of Zinc deficiency on plasma proteins in males and females

The mean plasma protein concentrations in the zinc deficient groups shown in Table 13a were significantly higher ($P < 0.01$) in males, with a mean of 8.68 g/100ml, than in female lambs, with a mean of 8.25 g/100ml. No significant difference was detected between the mean plasma protein concentration in pair-fed control male and female lambs.

Period 2

The mean plasma protein concentration, 6.41 g/ml of the zinc deficient group increased significantly ($P < 0.01$) during the six weeks to a mean of 9.39 g/100 ml, shown in week 12, Table 12b. Further, the mean plasma

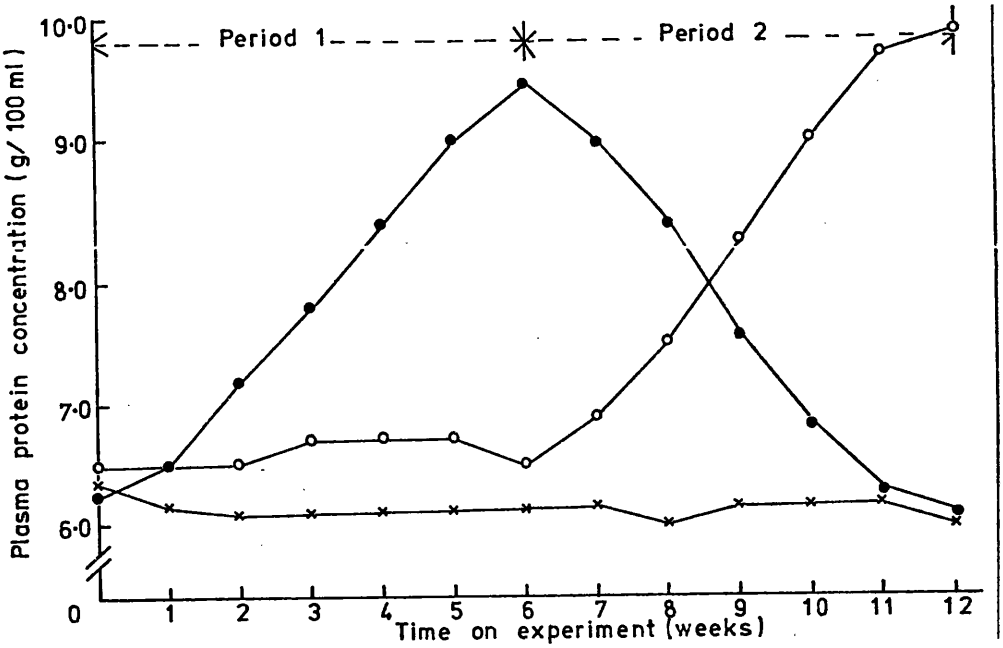


FIG. 10 MEAN PLASMA PROTEIN CONCENTRATION IN MALE LAMBS

●—●—● Zinc deficient in Period 1, Zinc supplemented in Period 2.

○—○—○ Ad lib control in Period 1, Zinc deficient in Period 2.

×—×—× Pair fed control in Period 1 and 2.

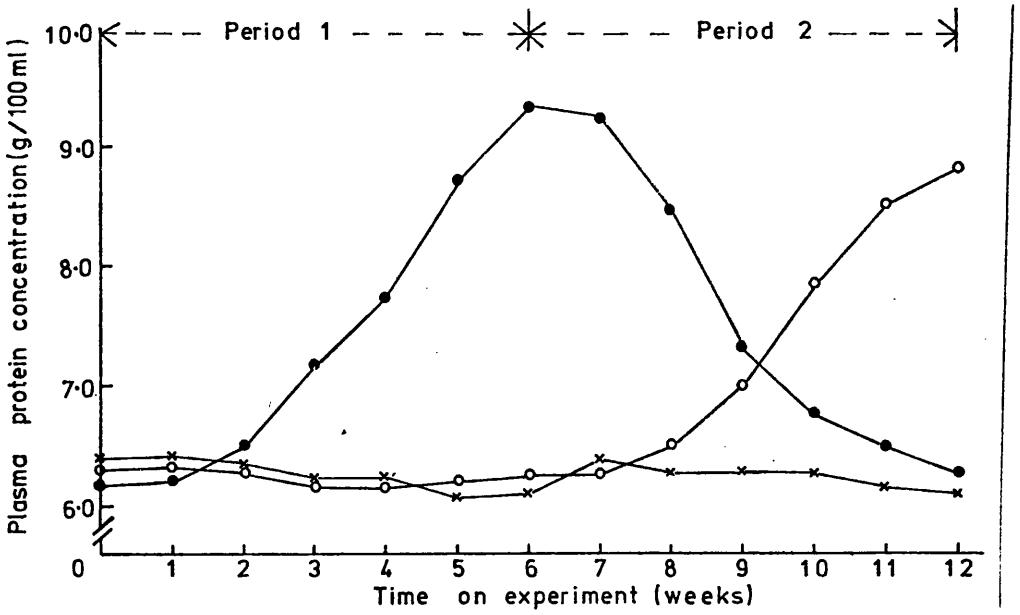


FIG. 11 MEAN PLASMA PROTEIN CONCENTRATION IN FEMALE LAMBS

●—●—● Zinc deficient in Period 1, Zinc supplemented in Period 2.

○—○—○ Ad lib control in Period 1, Zinc deficient in Period 2.

×—×—× Pair fed control in Period 1 and 2.

TABLES 12a AND 12b

Mean weekly plasma protein concentration (g/100 ml) of lambs on different dietary treatments in Periods 1 and 2

Table 12a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	7.54	6.42	6.19
4	8.09	6.39	6.17
5	8.80	6.44	6.17
6	9.43	6.41	6.17

Standard error ± 0.11

means calculated from 10 values

Table 12b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	7.65	7.64	6.17
10	8.40	6.93	6.17
11	9.08	6.39	6.20
12	9.39	6.18	6.18

Standard error ± 0.18

Means calculated from 10 values

TABLES 13a AND 13b

The effect of zinc deficiency on plasma protein in male and female lambs

Table 13a Period 1

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	8.68	6.60	6.11
Female	8.25	6.23	6.24

Standard error ± 0.10

Means calculated from 5 values

Table 13b Period 2

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	9.22	6.66	6.11
Female	8.04	6.92	6.26

Standard error ± 0.13

Means calculated from 5 values

protein concentration of the zinc deficient group for the whole period (8.63 g/100 ml) was significantly higher ($P < 0.001$) than the mean 6.18 g/100 ml for the pair-fed control group.

Effect of Zinc Deficiency on Plasma Proteins in Males and Females

The mean plasma protein concentrations of the zinc deficient groups shown in Table 13b were significantly higher ($P < 0.01$) in males (9.22 g/100 ml) than in female lambs (8.04 g/100 ml).

No significant difference was detected between plasma protein concentrations in pair-fed control male and female lambs.

Effect of Zinc Supplementation

The mean plasma protein concentration of 9.43 g/100 ml, in the ad lib control group (previously zinc deficient in Period 1) at the commencement of Period 2, decreased significantly ($P < 0.001$) after six weeks to 6.18 g/100 ml in week 12.

Therefore the means in Table 12b show that the effect of supplementing the diet of zinc deficient lambs with zinc was successful in reversing the raised plasma protein to the same value as in the pair-fed control group of 6.18 g/100ml in week 12. This result implies that the zinc supplemented in the diet was physiologically available and possibly controlled by a homeostatic mechanism in the plasma.

Plasma Albumin Concentration

The results are shown in Figure 12 and Tables 14a and 14b. It is seen that dietary deficiency of zinc caused the albumin concentration to decrease during the six weeks in Periods 1 and 2.

Period 1

The mean albumin concentration in the zinc deficient group decreased significantly ($P < 0.05$) during six weeks from 3.80 g/100 ml at the beginning of the period to 3.10 g/100 ml at week 6. Further, the mean albumin concentration of the zinc deficient group for the period, 3.24 g/100 ml, was significantly lower ($P < 0.05$) than 3.73 g/100 ml for the pair-fed control group.

Effect of Zinc Deficiency on Albumin in Males and Females

The results are shown in Table 15a. The mean albumin concentration 3.17 g/100 ml in zinc deficient male lambs was significantly lower ($P < 0.05$) than 3.31 g/100 ml for female lambs fed a similar diet. There was no significant difference between mean albumin concentration in male and female pair-fed control groups.

Period 2

The mean albumin concentration decreased significantly ($P < 0.01$) from 3.92 to 3.15 g/100 ml in the zinc deficient lambs during this period. Similarly in Period 1 the mean albumin concentration in zinc deficient groups for the period, 3.57 g/100 ml was significantly lower ($P < 0.05$) than 3.75 g/100 ml for the pair-fed control group.

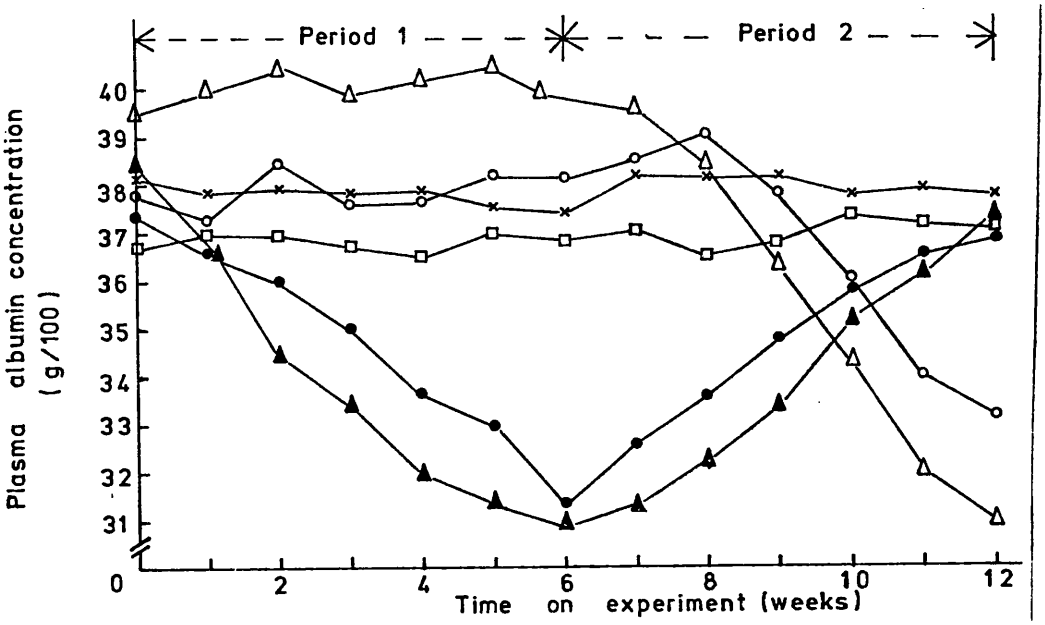


FIG. 12 MEAN PLASMA ALBUMIN CONCENTRATION

Zinc deficient males in Period 1, Zinc supplemented Ad lib in Period 2

Ad lib male control in Period 1, Zinc deficient in Period 2.

Pair fed male control in Periods 1 and 2.

Zinc deficient females in Period 1, Zinc supplemented Ad lib in Period 2.

Ad lib female control in Period 1, Zinc deficient in Period 2.

Pair fed female controls in Periods 1 and 2.

TABLES 14a AND 14b

Mean Plasma Albumin Concentration (g/100 ml) of lambs on different dietary treatments in Periods 1 and 2

Table 14a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	3.42	3.88	3.73
4	3.28	3.89	3.72
5	3.18	3.93	3.74
6	3.10	3.92	3.72

Standard error ± 0.05

Means calculated from 10 values

Table 14b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	3.71	3.38	3.75
10	3.83	3.53	3.75
11	3.31	3.65	3.77
12	3.15	3.72	3.75

Standard error ± 0.05

Means calculated from 10 values

TABLES 15a AND 15b

The effect of Zinc Deficiency on Plasma Albumin in Male and Female Lambs

Table 15a Period 1

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	3.17	4.03	3.68
Female	3.31	3.78	3.77

Standard error ± 0.03

Means calculated from 5 values

Table 15b Period 2

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	3.31	3.56	3.72
Female	3.68	3.58	3.78

Standard error ± 0.07

Means calculated from 5 values

Effect of Zinc Deficiency on Albumin in Males and Females

The results in Table 15b show that the effect of zinc deficiency was similar to Period 1. The mean albumin concentration of zinc deficient male lambs of 3.31 g/100 ml was significantly lower ($P < 0.05$) than in females, 3.68 g/100 ml, fed similar diets. There was no significant difference between mean albumin concentrations in male and female pair-fed control groups.

Effect of Zinc Supplementation

The mean albumin concentration 3.10 g/100 ml in the ad lib control group (previously zinc deficient) increased significantly ($P < 0.01$) after six weeks to 3.72 g/100 ml. The mean albumin concentration in week 6 of the pair-fed control group was 3.75 g/100 ml.

Plasma Globulin Concentration

Period 1

Figure 13 and Table 16a show that dietary deficiency of Zinc resulted in the globulin concentration increasing during the six weeks. This increase was significant ($P < 0.01$) between means in the Zinc deficient group from 2.35 g/100 ml at the start of the period, rising to 6.32 g/100 ml in week 6.

The mean globulin concentration, 5.22 g/100 ml for the Zinc deficient group was significantly higher ($P < 0.01$) than the 2.46 g/100 ml for the pair-fed control group.

Effect of Zinc deficiency on Plasma Globulin in Males and Females

These results are shown in Table 17a. The mean globulin concentration of Zinc deficient male lambs, 5.49 g/100 ml was significantly higher ($P < 0.01$) than the mean globulin concentration of 4.95 g/100 ml for females on this diet. No significant difference existed between the mean globulin concentration in pair-fed control male and female lambs.

Period 2

The effect of Zinc deficient diets on globulin concentration in this period is shown in Figure 13 and Table 16b and were similar to results in Period 1. The mean globulin increased significantly ($P < 0.01$) from 2.48 to 6.25 g/100 ml in six weeks.

The mean globulin concentration 5.08 g/100 ml for the Zinc deficient group was significantly higher ($P < 0.05$) than 2.44 g/100 ml for the pair-fed control group.

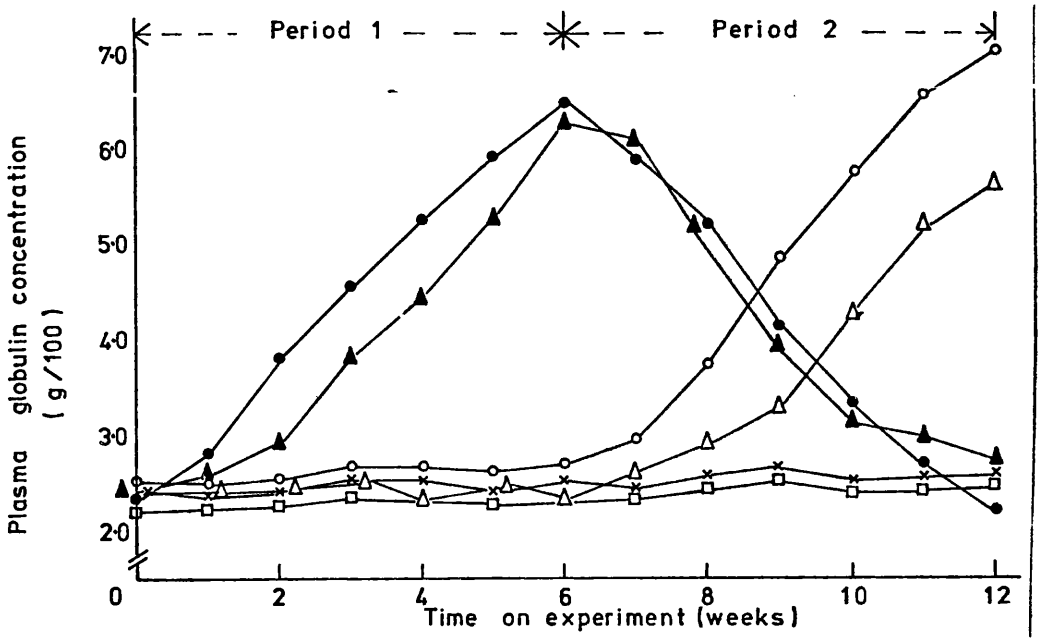


FIG. 13 MEAN PLASMA GLOBULIN CONCENTRATION

Zinc deficient males in Period 1, Zinc supplemented Ad lib in Period 2.

Ad lib male control in Period 1, Zinc deficient in Period 2.

Pair fed male control in Periods 1 and 2.

Zinc deficient females in Period 1, Zinc supplemented Ad lib in Period 2.

Ad lib female control in Period 1, Zinc deficient in Period 2.

Pair fed female control in Periods 1 and 2.

TABLES 16a AND 16B

Mean Weekly Globulin Concentration (g/100 ml) of Lambs on Different Dietary Treatments in Periods 1 and 2

Table 16a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	4.13	2.52	2.47
4	4.81	2.49	2.48
5	5.62	2.50	2.43
6	6.32	2.48	2.45

Standard error ± 0.08

Means calculated from 10 values

TABLE 16b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	3.96	4.27	2.44
10	4.35	3.39	2.44
11	5.76	2.73	2.45
12	6.25	2.46	2.44

Standard error ± 0.47

Means calculated from 10 values

TABLES 17a AND 17b

The Effect of Zinc Deficiency on Plasma Globulin in Male and Female Lambs

Table 17a Period 1

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	5.49	2.57	2.45
Female	4.95	2.43	2.46

Standard error ± 0.06

Means calculated from 5 values

Table 17b Period 2

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	5.91	3.09	2.43
Female	4.25	3.33	2.45

Standard error ± 0.33

Means calculated from 5 values

Effect of Zinc Supplementation

The response of the plasma globulin in lambs fed a Zinc supplemented diet after six weeks on a Zinc deficient diet is shown by the ad lib group (previously zinc deficient group). The mean globulin concentration decreased significantly ($P < 0.01$) from 6.32 to 2.46 g/100 ml in six weeks; thus this globulin concentration had returned to mean pair-fed control value of 2.44 g/100 ml.

Effect of Zinc Deficiency on Plasma Globulin in Males and Females

The results are shown in Table 17b. The effect is similar to results in Period 1. The mean globulin concentration 5.91 g/100 ml of Zinc deficient males was significantly higher ($P < 0.05$) than the mean globulin concentration 4.25 g/100 ml for females on this diet. The pair-fed control male and female lambs were not significantly different in their globulin concentrations.

Plasma α_1- and α_2-Globulin Concentrations

The effect of Zinc deficiency on these proteins (Tables 18a and 18b; 20a and 20b) was similar to previous results for total globulin. Both globulins in the Zinc deficient groups increased in concentration during Periods 1 and 2. A trend towards higher concentrations in males rather than females was identified in Period 1 but not in Period 2.

Period 1

The α_1-globulin concentration of the Zinc deficient group increased significantly ($P < 0.01$) during six weeks from a mean of 0.30 g/100 ml to 1.24 g/100 ml.

The α_2-globulin concentration of the Zinc deficient group however showed no significant difference during six weeks between means of 0.70 g/100 ml and 0.74 g/100 ml.

The mean α_1-globulin of 0.98 g/100 ml of the Zinc deficient group was significantly higher ($P < 0.01$) than the 0.20 g/100 ml of the pair-fed control group.

Similarly, the mean α_2-globulin 1.12 g/100 ml of the Zinc deficient group was significantly higher ($P < 0.01$) than 0.43 g/100 ml of the pair-fed control group.

Effect of Zinc Deficiency on α_1- and α_2-Globulins in Males and Females

The results are shown in Tables 19a, 19b, 21a and 21b. The mean concentrations of these proteins in the Zinc deficient groups were significantly higher ($P < 0.01$) in the males than in females.

Period 2

The mean α_1- and α_2-globulin concentrations in Zinc deficient groups increased significantly ($P < 0.01$)

TABLES 18a AND 18b

The Mean Weekly α1-Globulin Concentration (g/100 ml) of Lambs on Different Dietary Treatments in Periods 1 and 2

Table 18a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	0.69	0.25	0.20
4	0.90	0.21	0.21
5	1.09	0.23	0.21
6	1.24	0.21	0.20

Standard error ± 0.09

Means calculated from 10 values

Table 18b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	0.47	0.69	0.21
10	1.30	0.51	0.22
11	0.81	0.33	0.22
12	0.92	0.31	0.21

Standard error ± 0.20

Means calculated from 10 values

TABLES 19a AND 19b

The Effect of Zinc Deficiency on α1-Globulin in Male and Female Lambs

Table 19a Period 1

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	1.28	0.25	0.21
Female	0.68	0.21	0.20

Standard error ± 0.07

Means calculated from 5 values

Table 19b Period 2

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	0.89	0.55	0.23
Female	0.86	0.37	0.20

Standard error ± 0.14

Means calculated from 5 values

TABLES 20a AND 20b

The Mean Weekly α2-Globulin Concentration (g/100 ml) of Lambs on Different Dietary Treatments in Periods 1 and 2

Table 20a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	0.61	0.56	0.44
4	0.71	0.53	0.46
5	0.71	0.52	0.44
6	0.74	0.50	0.42

Standard error ± 0.05

Means calculated from 10 values

Table 20b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	0.73	0.61	0.41
10	1.71	0.63	0.45
11	1.01	0.54	0.44
12	1.06	0.52	0.43

Standard error ± 0.25

Means calculated from 10 values

TABLES 21a AND 21b

The Effect of Zinc Deficiency on α2-Globulin in Male and Female Lambs

Table 21a Period 1

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	0.61	0.75	0.42
Female	0.45	0.63	0.45

Standard error ± 0.02

Means calculated from 5 values

Table 21b Period 2

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	1.21	0.68	0.41
Female	1.04	0.46	0.45

Standard error ± 0.18

Means calculated from 5 values

during six weeks from 0.21 to 0.92 g/100 ml for α1-globulin and from 0.50 to 1.06 g/100 ml for 2-globulin. The α1- and α2-globulin concentrations in the corresponding pair-fed control groups remained constant at 0.21 and 0.44 g/100 ml respectively.

Effect of Zinc Supplementation

Mean α1- and α2-globulin concentrations, 1.24 and 0.74 g/100 ml respectively at the commencement of Period 2 decreased significantly ($P<0.01$) in the ad lib control group (previously Zinc deficient in Period 1) down to 0.31 and 0.52 g/100 ml respectively after six weeks of consuming Zinc supplemented diet. The corresponding α1- and α2-globulin concentrations in the pair-fed control groups at Week 6 were 0.21 and 0.43 g/100 ml respectively.

Effect of Zinc Deficiency on α1- and α2-Globulins in Males and Females

The mean concentrations of these proteins showed higher trends in the males than in females. The male α1-globulin was 3% higher than females, whilst male α2-globulin was 16% higher than females.

Plasma β- and γ-Globulin Concentrations

The results for mean concentrations of β- and γ-globulins are shown in Tables 22a, 22b; and 24a and 24b respectively.

Period 1

Results in Tables 22a and 24a show that both the mean β- and γ-globulin concentration of the Zinc deficient group increased significantly ($P < 0.05$) during six weeks, the mean concentration of β-globulin increased from 0.67 g/100 ml to 1.98 g/100 ml; the mean γ-globulin concentration increased from 0.75 g/100 ml to 2.37 g/100 ml.

Further, both the β- and γ-globulin concentrations of the Zinc deficient group, 1.65 g/100 ml and 1.90 g/100 ml, were significantly higher ($P < 0.001$) than the corresponding concentrations 0.80 g/100 ml and 1.02 g/100 ml for β- and γ-globulins respectively in the pair-fed control group.

Effect of Zinc Deficiency on β- and γ-globulins in Males and Females

The results in Tables 23a and 25a show that there was no significant difference between male and female Zinc deficient lambs in either β- or γ-globulin concentration.

Period 2

Results in Tables 22b and 24b show that the mean β- and γ-globulin concentrations of the Zinc deficient group increased significantly ($P < 0.05$) during six weeks; the mean β-globulin increased from 0.78 to 1.92 g/100 ml, and the mean γ-globulin concentration increased from 0.99 to 2.39 g/100 ml.

TABLES 22a AND 22b

The mean weekly β-Globulin Concentration (g/100 ml) of Lambs on Different Dietary Treatments in Periods 1 and 2

Table 22a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	1.32	0.75	0.82
4	1.52	0.73	0.79
5	1.79	0.76	0.78
6	1.98	0.78	0.80

Standard error ± 0.07

Means calculated from 10 values

Table 22b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	1.24	1.38	0.81
10	1.95	0.99	0.77
11	1.77	0.81	0.76
12	1.92	0.69	0.80

Standard error ± 0.16

Means calculated from 10 values

TABLES 23a AND 23b

The Effect of Zinc Deficiency on β-Globulin in Male and Female Lambs

Table 23a Period 1

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	1.61	0.72	0.78
Female	1.69	0.78	0.82

Standard error ± 0.05

Means calculated from 5 values

Table 23b Period 2

Sex	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
Male	1.72	0.83	0.75
Female	1.72	1.10	0.82

Standard error ± 0.10

Means calculated from 5 values

TABLES 24a AND 24b

Mean Weekly γ-Globulin Concentration (g/100 ml) of Lambs on Different Dietary Treatments in Periods 1 and 2

Table 24a Period 1

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
3	1.51	0.96	1.01
4	1.69	1.02	1.02
5	2.02	1.01	1.01
6	2.37	0.99	1.03

Standard error $\pm$ 0.05

Means calculated from 10 values

Table 24b Period 2

Weeks of experimental period	Treatments		
	Zinc deficient group	Ad libitum control group	Pair fed control group
9	1.52	1.66	1.02
10	2.47	1.24	1.01
11	2.19	1.04	1.03
12	2.39	0.94	1.00

Standard error $\pm$ 0.22

Means calculated from 10 values

TABLES 25a AND 25b

The Effect of Zinc Deficiency on γ-Globulin in Male and Female Lambs

Table 25a Period 1

Sex	Treatments		
	Zinc Deficient group	Ad libitum control group	Pair fed control group
Male	1.84	1.00	1.04
Female	1.95	0.99	1.00

Standard error ± 0.03

Means calculated from 5 values

Table 25b Period 2

Sex	Treatments		
	Zinc Deficient group	Ad libitum control group	Pair fed control group
Male	2.11	0.99	1.03
Female	2.17	1.45	1.00

Standard error ± 0.15

Means calculated from 5 values

Over the last four weeks of the period the mean β- and γ-globulin concentrations of the Zinc deficient group were 1.72 and 2.14 g/100 ml respectivcly. This was significantly higher ($P<0.05$) than the corresponding concentrations 0.78 and 1.01 g/100 ml of these globulins respectively in the pair-fed control group.

Effect of Zinc Deficiency on β- and γ-globulins in Males and Females

The results in Tables 23b and 25 b show that there was no significant difference between male and female Zinc deficient lambs in either β- or γ-globulin concentration.

Effect of Zinc Supplementation

The mean β- and γ-globulin concentrations of 1.98 and 2.37 g/100 ml respectively, of the ad lib control group (previously Zinc deficient in Period 1) at the commencement of Period 2, decreased significantly ($P<0.01$) after six weeks. The β-globulin decreased to 0.69 g/100 ml, whilst the γ-globulin decreased to 0.94 g/100 ml.

The corresponding β- and γ-globulin concentrations in the pair-fed control groups at Week 6 were 0.80 and 1.00 f/100 ml respectively.

CHAPTER 8

An Investigation of Bound and Unbound Plasma Zinc by Ultrafiltration

Introduction

The ultrafiltration technique used to measure the amount of plasma bound Zinc has been described in Chapter 4. This method retained proteins with molecular weights greater than 500 which were subsequently analyzed for Zinc by atomic absorption spectrophotometry. This quantity of Zinc was the bound Zinc.

The general design of this experiment was similar to previous experiments, with the exception that only male lambs were used.

Five lambs were allocated to each of the groups A and B. Group A was fed a Zinc deficient diet for six weeks (Period 1) and then fed a Zinc supplemented diet for the subsequent six weeks (Period 2). Group B was fed a Zinc supplemented diet for the first six weeks (Period 1) followed by a Zinc deficient diet for the subsequent six weeks (Period 2). Therefore each group had a control group during each six week experimental period.

Results

The amount and proportion of the total plasma Zinc bound to plasma proteins (molecular weight >500) in group B lambs which were fed Zinc supplement diets for six weeks, is shown in Table 26 and Period 1 of Figure 14. The mean quantity of protein bound Zinc in this group for six weeks was 1.05 µg.Zn/ml. This was a mean 92.4 per cent of the total plasma Zinc. Therefore, by calculation, the proportion of unbound plasma Zinc was a mean 7.6 per cent.

The effect of feeding a Zinc deficient diet on the amount and proportion of bound and unbound plasma Zinc is shown in Period 2, Figure 14 and Table 27. The amount of protein bound Zinc decreased rapidly by 22.5 per cent of total plasma Zinc during the first week of feeding the Zinc deficient diet; at the beginning of the period, the bound Zinc was 1.05 µg.Zn/ml, corresponding to 92.1 per cent of bound Zinc in total plasma Zinc, which decreased to 0.16 µg.Zn/ml, 69.6 per cent of total plasma Zinc, during the first week of feeding the deficient diet. The amount of protein bound Zinc continued to decrease for the following three weeks, reaching the lowest amount, 0.07 µg.Zn/ml, in week 10. During weeks 11 and 12, however, there was a trend showing protein bound Zinc increasing. This increase did not reach levels of statistical significance within the time of the experiment.

The amount of unbound plasma Zinc shown in Figure 14 and Tables 26 and 27 was relatively unchanged throughout Periods 1 and 2. The changeover in the diet of the lambs

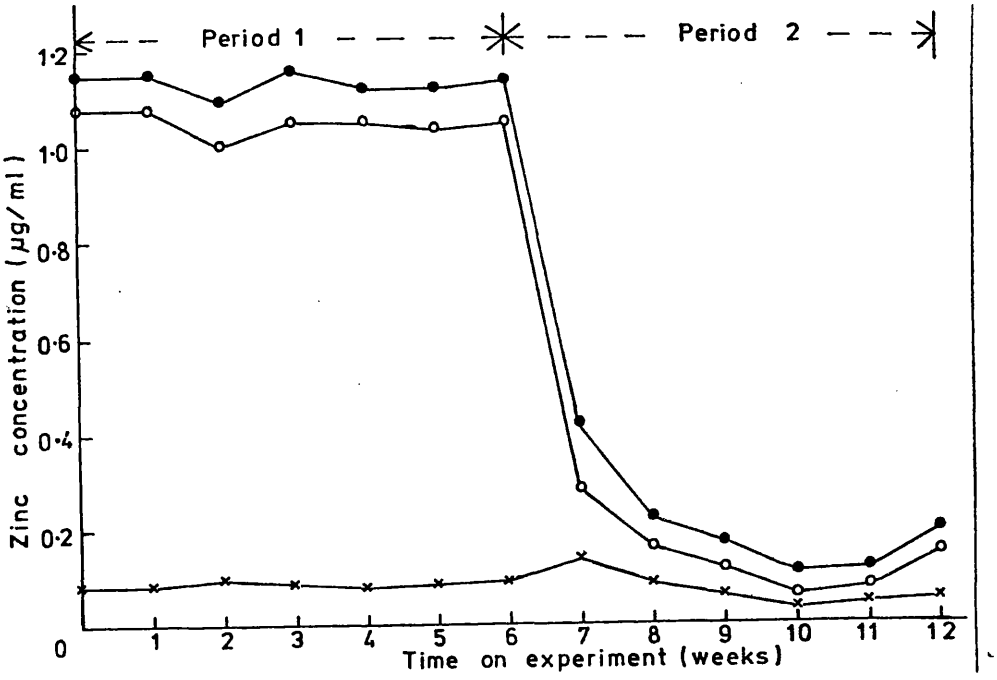


FIG. 14 GROUP B EFFECT OF ZINC SUPPLEMENTATION IN WEEKS 1-6 ON TOTAL PLASMA ZINC, BOUND AND UNBOUND ZINC.

This graph also shows the effect of Zinc deficiency in weeks 6-12 on total plasma Zinc, bound and unbound zinc.

●—●—● Total plasma Zinc concentration

o—o—o Plasma bound Zinc

×—×—× Unbound plasma Zinc

TABLE 26

The Effect of Zinc Supplementation on Total Zinc, Protein-Bound Zinc and Unbound Zinc in Male Lamb Plasma

Group B

Weeks	Total plasma Zinc µg/ml	Protein bound Zinc		Unbound Plasma Zinc	
		µg/ml	Percentage of total Zinc	µg/ml	Percentage of total Zinc
0	1.15 ± 0.03	1.07 ± 0.05	93.0	0.08 ± 0.05	7.0
1	1.15 ± 0.02	1.07 ± 0.05	93.0	0.08 ± 0.04	7.0
2	1.10 ± 0.02	1.00 ± 0.02	91.0	0.10 ± 0.06	9.0
3	1.16 ± 0.03	1.07 ± 0.04	92.2	0.09 ± 0.05	7.8
4	1.13 ± 0.02	1.05 ± 0.04	92.9	0.08 ± 0.04	7.1
5	1.12 ± 0.02	1.04 ± 0.03	92.9	0.08 ± 0.04	7.1
6	1.14 ± 0.02	1.05 ± 0.04	92.1	0.09 ± 0.04	7.9
Means	1.14	1.05	92.4	0.08	7.6

TABLE 27

The Effect of Zinc Deficiency on Total Zinc, Protein-Bound Zinc and Unbound Zinc

Group B

Weeks	Total plasma Zinc µg/ml	Protein bound Zinc		Unbound Plasma Zinc	
		µg/ml	Percentage of total Zinc	µg/ml	Percentage of total Zinc
7	0.42 ± 0.05	0.29 ± 0.06	69.0	0.11 ± 0.06	26.2
8	0.23 ± 0.04	0.16 ± 0.05	69.6	0.07 ± 0.03	30.4
9	0.18 ± 0.02	0.13 ± 0.05	72.2	0.05 ± 0.04	27.8
10	0.11 ± 0.03	0.07 ± 0.03	63.6	0.04 ± 0.04	36.4
11	0.12 ± 0.04	0.08 ± 0.04	66.7	0.04 ± 0.04	33.3
12	0.20 ± 0.03	0.15 ± 0.07	75.0	0.05 ± 0.05	25.0

from Zinc supplemented in Period 1 to Zinc deficient diet is reflected, to a slight extent, in the amount of unbound Zinc shown in Figure 14. A slight decrease in unbound Zinc concentration occurred during the first few weeks of Period 2, subsequently a slight rise was apparent before the end of this Period.

The results of feeding a Zinc deficient diet to lambs in group A confirmed in the main, the results for similarly treated lambs in group B. These results for group A are shown in Period 1 of Figure 15. The protein bound Zinc decreased rapidly during the first week by 17.0 per cent of the total plasma Zinc.

In the subsequent weeks, the protein bound Zinc in group A lambs appeared to adapt sooner to the Zinc deficient diet and did not reach the low values compared to lambs in group B.

When the lambs in group A were fed the Zinc supplemented diet in Period 2 shown in Fig. 15, Tables 28 & 29 the protein bound Zinc increased rapidly in the first week but more slowly during subsequent weeks achieving values similar to controls after five weeks.

The amount of unbound Zinc remained relatively unchanged during Periods 1 and 2.

In both groups A and B, it was noticeable (Figures 14 and 15) that both the plasma bound and the total plasma Zinc levels, showed similar trends during the experimental periods.

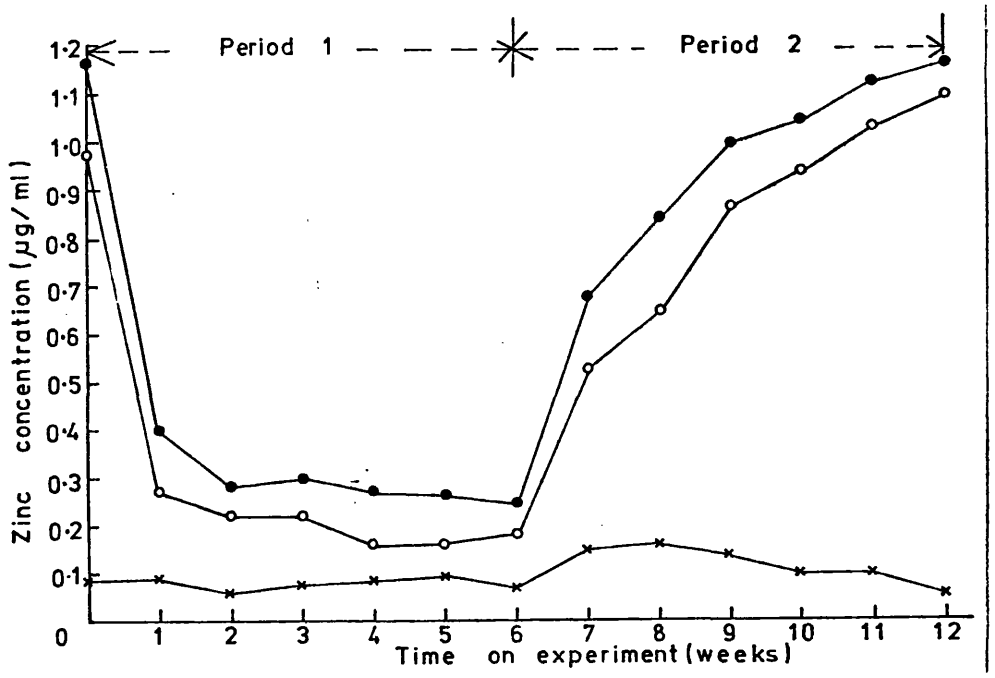


FIG. 15 GROUP A EFFECT OF ZINC DEFICIENCY IN WEEKS 1-6 ON TOTAL PLASMA ZINC, BOUND AND UNBOUND ZINC.

This graph also shows the effect of Zinc supplement in weeks 6-12 on total plasma zinc, bound and unbound Zinc.

●—●—● Total plasma zinc concentration

o—o—o Plasma bound zinc.

×—×—× Unbound plasma zinc.

TABLE 28

The Effect of Zinc Deficiency on Total Zinc, Protein-Bound Zinc and Unbound Zinc in Male Lamb Plasma

Group A

Weeks	Total plasma Zinc µg/ml	Protein bound Zinc		Unbound plasma Zinc	
		µg/ml	Percentage of total Zinc	µg/ml	Percentage of total Zinc
0	1.16 ± 0.04	1.07 ± 0.06	92.2	0.09 ± 0.03	7.8
1	0.40 ± 0.02	0.30 ± 0.04	75.0	0.09 ± 0.05	22.5
2	0.28 ± 0.02	0.22 ± 0.05	78.6	0.06 ± 0.05	21.4
3	0.30 ± 0.02	0.22 ± 0.05	73.3	0.08 ± 0.04	26.7
4	0.27 ± 0.03	0.18 ± 0.05	66.7	0.09 ± 0.05	33.3
5	0.26 ± 0.03	0.16 ± 0.04	61.5	0.10 ± 0.06	38.5
6	0.25 ± 0.02	0.18 ± 0.03	72.0	0.07 ± 0.05	28.0

TABLE 29

The Effect of Zinc Supplementation on Total Zinc, Protein-Bound Zinc and Unbound Zinc

Group A

Weeks	Total plasma Zinc µg/ml	Protein bound Zinc		Unbound plasma Zinc	
		µg/ml	Percentage of total Zinc	µg/ml	Percentage of total Zinc
7	0.69 ± 0.02	0.54 ± 0.04	78.3	0.15 ± 0.07	21.7
8	0.85 ± 0.03	0.69 ± 0.04	81.2	0.16 ± 0.06	18.8
9	1.00 ± 0.06	0.86 ± 0.05	86.0	0.14 ± 0.06	14.0
10	1.05 ± 0.05	0.95 ± 0.04	90.5	0.10 ± 0.05	9.5
11	1.13 ± 0.04	1.03 ± 0.06	91.2	0.10 ± 0.04	8.8
12	1.16 ± 0.05	1.10 ± 0.06	94.8	0.06 ± 0.04	5.2

CHAPTER 9

Investigation of Zinc Binding Plasma Proteins

Introduction

The main objective of the following experiments was to locate and characterize the Zinc binding proteins in plasma of Zinc deficient lambs. From the results seen in Chapter 8 it is reasonable to assume that a great proportion of plasma Zinc was in the bound state, and probably bound to plasma proteins.

The methods used to separate the Zinc binding proteins are described in Chapter 4; these were ion-exchange and gel filtration chromatography. After the plasma proteins fractions had been seperated by ion-exchange, the fractions containing Zinc, fraction numbers 55 - 90 inclusive shown in Figure 16, were pooled and further separated by gel-filtration.

The methods used to characterize the Zinc binding proteins were: disc gel electrophoresis, molecular weight determinations, and the trypsin-protein esterase activity. All these methods are described in Chapter 4. Molecular weight determinations were confirmed by the ultracentrifugation method of Schonenberger et al (1968).

Results

A typical separation of the plasma proteins of Zinc supplemented lambs by ion-exchange chromatography is shown in Figure 16. After analysis of all fractions by Atomic absorption spectroscopy, only fractions 55 to 90 inclusive, were found to contain detectable amounts of Zinc.

These Zinc containing protein fractions were pooled and further separated by gel-filtration. A typical separation pattern is shown in Figure 17. The Zinc was located in two protein peaks. The first protein to be eluted from the column in fractions 11 to 14 contained a smaller amount of protein, a mean 0.24 $\pm$ 0.06 g/100 ml, than the second eluted protein in fractions 15 to 21 which contained a mean 3.5 $\pm$ 0.15 g/100 ml. The corresponding Zinc content of these fractions showed that the first eluted protein contained a mean of 25 $\pm$ 0.05 µg.Zn/100 ml; this was 66 per cent less than the mean Zinc content, 75 $\pm$ 0.07 µg.Zn/100 ml of the second eluted protein.

The eluted fractions from the gel-filtration column were tested on polyacrylamide gel electrophoresis at pH 8.1. The results (Figure 18) show that samples from both the first and second eluted protein fractions, corresponding to fraction numbers 11 to 14, and 15 to 21 in Figure 17 respectively, contained two seperate protein bands. A distinct slow moving protein band is shown in gel 1 and a faster protein band in gel 3. The migration characteristics of these protein bands agreed with those published by Maurer (1971) and Smith (1968) for α2-macroglobulin in gel 1, and for albumin in gel 3.

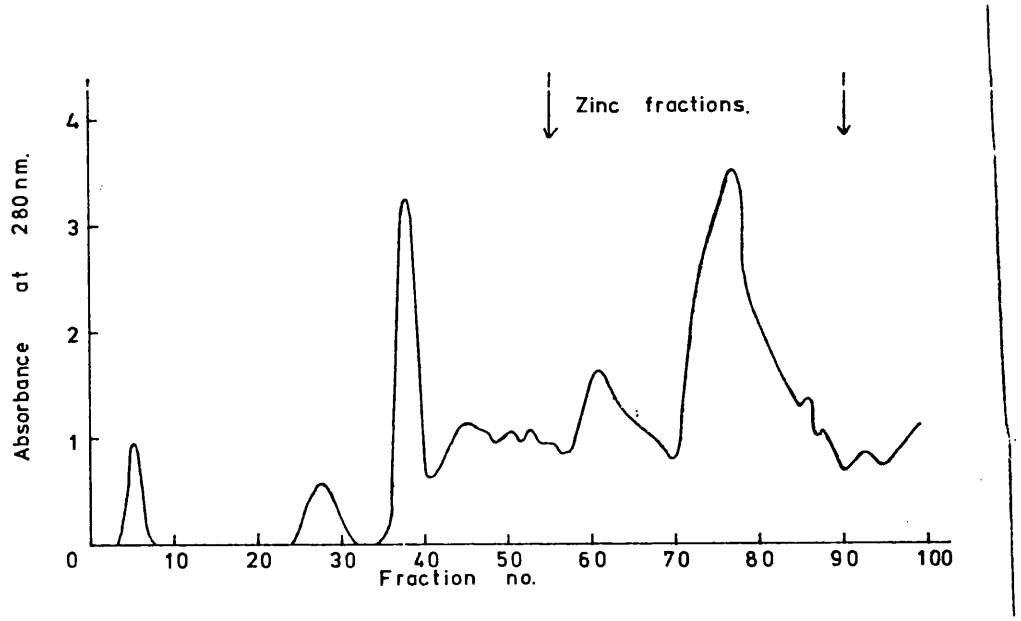


FIG. 16 SEPERATION OF ZINC CONTAINING PROTEINS IN LAMB PLASMA

Ion-exchange chromatography was usedin 2.6 x 40 cm. columns of D.E.A.E.-cellulose. The columns were equilibriated with 0.025M Succinic acid and 0.2M-tris hydrochloride buffer, pH 8.6.
Each fraction = 10 ml.
Zinc containing fractions were No. 55-90.

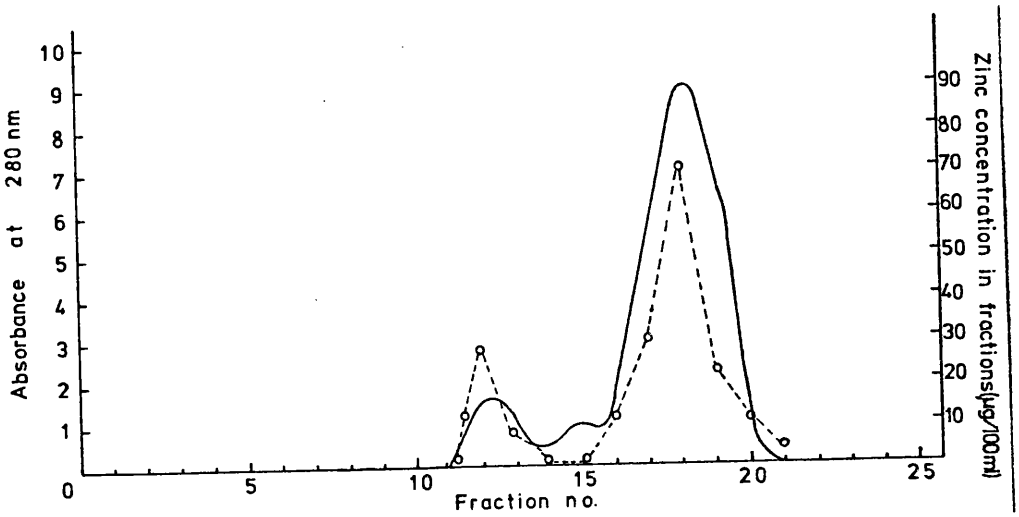


FIG. 17 SEPERATION OF POOLED ZINC CONTAINING FRACTIONS BY GEL FILTRATION

Fraction No. 55-90 inclusive from the ion-exchange chromatography were pooled and seperated by Gel-filtration using Sephadex G-100 on 2.6 x 40 cm. columns, equilibriated with 0.1M-tris hydrochloride buffer, pH 8.2. Each fraction = 5 ml.

o--o--o Zinc concentration

——— Protein concentration

Polyacrylamide Disc Gel Electrophoresis of Proteins in Eluate Fractions from Gel Filtration and Ion-Exchange Chromatography

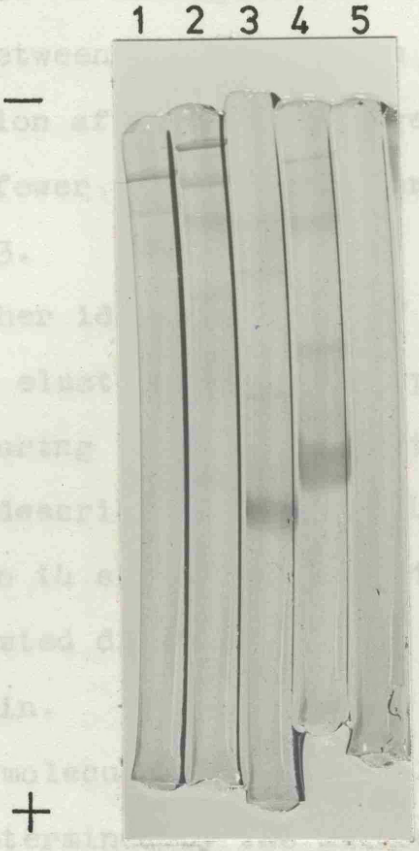


FIG. 18 All samples were placed on gels at negative end and proteins migrated towards the positive end.

Gel 1 shows separation of protein from pooled fractions 11 to 14 (Fig. 17) eluted from gel filtration column.

Gel 2 shows separation of proteins from pooled fractions 55 to 70 (Fig. 16) eluted from ion-exchange column.

Gel 3 shows separation of proteins from pooled fractions 15 to 21 (Fig. 17) eluted from gel filtration column.

Gel 4 shows separation of proteins from pooled fractions 70 to 90 (Fig. 16) eluted from ion-exchange column.

Gel 5 contained sample of eluant from fraction 23 of gel filtration column.

These two proteins are also shown in Figure 18 separated from ion-exchange chromatography eluate fractions before they were further purified by gel-filtration. Gel 2 and 4 show α2-macroglobulin and albumin respectively. The contrast between the seperation of these two proteins by gel filtration after ion-exchange chromatography, is very marked; fewer contaminating protein bands are present in gels 1 and 3.

Further identification of α2-macroglobulin in gel-filtration eluates was made by pooling eight protein bands and measuring the trypsin-protein esterase activity by the method described in Chapter 4. The pooled eluted fractions 11 to 14 shown in Figure 17 from the gel-filtration column were tested directly to show the presence of α2-macroglobulin.

The molecular weight of α2-macroglobulin and for albumin was determined by the method described in Chapter 4. The molecular weight of α2-macroglobulin was 790,000; and of albumin was 60,500.

The molecular weight of lamb or sheep α2-macroglobulin has not previously been found by the author in any published work. This present result, however, is in close agreement with the molecular weight determined by Schönenberger (1968) for α2-macroglobulin in human plasma. The molecular weight of albumin as determined in this work is also in close agreement with the molecular weight for human and bovine serum albumin determined by Edsall et al (1954).

CHAPTER 10

Investigation of Dietary Zinc Deficiency on Plasma Bound Zinc

Introduction

It was the intention to study the effect of a Zinc deficient diet on the proportion of plasma bound Zinc and on the distribution of Zinc between the different binding proteins.

The design of this experiment was similar to the previous outline described in Chapter 4. Male and female lambs were pair-fed and the investigation conducted for six weeks. This experiment was conducted in March of three consecutive years.

Plasma samples were taken, and the previously identified Zinc binding plasma proteins, albumin and α2-macroglobulin, were seperated by methods of ion-exchange and gel-filtration chromatography which are described in Chapter 4. These fractions were analyzed for proteins by the method of Lowry et al (1951) with modifications by Reider (1959), and for Zinc by Atomic absorption spectrophotometry. These methods have also been described in Chapter 4.

The amount of Zinc bound to α2-macroglobulin and albumin fractions was calculated and expressed as g.atoms Zinc per mole protein. The molecular weights of the proteins used for this calculation were determined as described in Chapter 4 and recorded in Chapter 9.

Results

Little difference was observed between the results for the pair-fed and ad libitum control groups, and therefore only the results of the pair-fed control group are presented in the following tables for comparison with the Zinc deficient group.

The results are presented to show the effect of dietary Zinc deficiency on the concentration of albumin and α_2-macroglobulin. The Zinc concentration of these fractions is expressed in two ways. Firstly, as µg.Zinc per 100 ml plasma in Table 32 and 33 respectively, and secondly, as g.atoms Zinc per mole of the respective protein, in Table 34 and 35. Finally, the amount of unbound Zinc is presented in Table 39.

Concentration of Albumin

The mean albumin concentrations in the Zinc deficient groups, shown in Table 30, decreased significantly ($P < 0.01$) from 3.6 to 2.4 g.Zn./100 ml in males and from 3.4 to 2.6 g.Zn./100 ml in females during the six week experimental period. This decrease in albumin concentration however, when compared with the control group, occurred during the last three weeks of the period. Therefore, dietary Zinc deficiency had no apparent effect on the albumin concentration during the first three weeks of consuming the diet.

TABLE 30

Concentration (g/100 ml) of Albumin Fractions
in Male and Female Lambs
Mean of Five Lambs ±S.E.

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	3.6 ± 0.2 NS	3.5 ± 0.3	3.7 ± 0.3 NS	3.5 ± 0.4
1	3.4 ± 0.2 NS	3.3 ± 0.2	3.4 ± 0.3 NS	3.4 ± 0.3
2	3.3 ± 0.2 NS	3.0 ± 0.3	3.2 ± 0.3 NS	3.4 ± 0.2
3	3.1 ± 0.3 NS	3.2 ± 0.2	3.1 ± 0.3 NS	3.4 ± 0.3
4	2.8 ± 0.2 *	3.2 ± 0.3	2.8 ± 0.3 *	3.4 ± 0.3
5	2.5 ± 0.3 *	3.0 ± 0.2	2.7 ± 0.2 *	3.4 ± 0.2
6	2.5 ± 0.2 *	3.0 ± 0.2	2.6 ± 0.3 *	3.5 ± 0.3

Compared with control of the same sex

* P $<$ 0.01

NS - Not significant

Concentration of α2-macroglobulin

The mean α2-macroglobulin concentrations in the Zinc deficient groups, shown in Table 31, increased significantly ($P < 0.01$) during the six week period from 0.23 to 0.36 g/100 ml in males and from 0.24 to 0.32 g/100 ml in females.

Zinc Concentration in the Albumin Fraction

The mean Zinc concentration of the albumin fraction in Zinc deficient groups shown in Table 32, decreased significantly ($P < 0.01$) during the first week from 70 to 10 µgZn./100 ml in males, and from 76 to 12 µgZn./100 ml in females. The low Zinc concentrations were reasonably constant for the remaining five weeks of the period.

Zinc Concentration in the α2-macroglobulin Fraction

The mean Zinc concentration of the α2-macroglobulin fraction in Zinc deficient groups shown in Table 33, decreased significantly ($P < 0.01$) during the six weeks from a mean of 24 µgZn./100 ml at the beginning of the period to 7.5 µgZn./100 ml in week 6.

A comparison of the means shown in Table 33 between the male and female Zinc deficient and pair-fed groups, showed that the Zinc concentration of the Zinc deficient females did not decrease significantly ($P < 0.01$) until week 4 of the period. In the male Zinc deficient group, however, the Zinc concentration decreased significantly ($P < 0.01$) in week 2.

It must be noted therefore that Zinc deficient lambs lost Zinc from their α2-macroglobulin fractions much later than Zinc was lost from the albumin fractions.

TABLE 31

Concentration (g/100 ml) of ∝2-macroglobulin fractions in Male and Female Lambs
Mean of Five Lambs ±S.E.

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	0.23 ± 0.05 NS	0.24 ± 0.05	0.24 ± 0.04 NS	0.25 ± 0.03
1	0.25 ± 0.07 NS	0.26 ± 0.05	0.26 ± 0.04 NS	0.27 ± 0.04
2	0.26 ± 0.04 NS	0.27 ± 0.06	0.27 ± 0.04 NS	0.25 ± 0.04
3	0.28 ± 0.04 NS	0.24 ± 0.06	0.29 ± 0.05 NS	0.26 ± 0.06
4	0.31 ± 0.08 *	0.24 ± 0.05	0.31 ± 0.06 *	0.24 ± 0.05
5	0.33 ± 0.07 *	0.24 ± 0.05	0.32 ± 0.05 *	0.25 ± 0.05
6	0.36 ± 0.07 *	0.24 ± 0.05	0.34 ± 0.06 *	0.24 ± 0.05

Compared with control of the same sex

*P $\leq$ 0.01

NS - Not significant

TABLE 32

Zinc concentration (µg/100 ml) of Albumin Fractions in Male and Female Lambs
Mean of Five Lambs ±S.E.

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	70 ± 3 NS	75 ± 4	76 ± 4 NS	75 ± 3
1	10 ± 3 *	69 ± 4	12 ± 3 *	77 ± 4
2	10 ± 2 *	67 ± 3	8 ± 3 *	76 ± 4
3	10 ± 2 *	66 ± 3	8 ± 3 *	75 ± 4
4	10 ± 3 *	67 ± 3	10 ± 2 *	74 ± 3
5	10 ± 3 *	68 ± 3	12 ± 3 *	76 ± 4
6	8 ± 4 *	65 ± 3	15 ± 3 *	74 ± 4

Compared with control of the same sex

*P <0.01

NS - Not significant

TABLE 33

Zinc concentration (ug/100 ml) of α2-macroglobulin fractions in Male and Female Lambs
Mean of Five Lambs ±S.E.

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	28 ± 3 NS	25 ± 3	20 ± 3 NS	23 ± 4
1	28 ± 3 NS	28 ± 3	18 ± 3 NS	22 ± 4
2	15 ± 3 *	25 ± 3	20 ± 2 NS	22 ± 4
3	15 ± 2 *	23 ± 4	20 ± 2 NS	24 ± 3
4	10 ± 2 *	26 ± 3	18 ± 2 *	27 ± 4
5	10 ± 2 *	28 ± 4	8 ± 3 *	23 ± 3
6	10 ± 2 *	26 ± 4	5 ± 2 *	23 ± 2

Compared with control of the same sex

*P <0.0

NS - not significant

Albumin - Bound Zinc

Table 34 and Figure 19 show that the amount of Albumin bound Zinc rapidly decreased in the Zinc deficient groups during the first week. In the control group, however, the albumin bound Zinc remained reasonably constant. In the males there was an overall decrease over the six weeks from 0.018 to 0.003 g.atoms Zinc/mole albumin. In the females there was an overall decrease from 0.020 to 0.006 g.atoms Zinc/mole albumin.

It must be noted that the low values for albumin bound Zinc were maintained for the remaining five weeks of the experiment.

Table 35 shows the proportion of the total plasma Zinc which was bound by albumin. The total plasma Zinc during this period is shown in Table 36. During the first week of consuming a Zinc deficient diet, the percentage of the total plasma Zinc decreased from a mean of 63.0 to 29.0 per cent.

The percentage of the total plasma Zinc which was bound by albumin, remained low in the males for the remaining five weeks of the period; in the females, however, during the last three weeks of the period there was a trend showing a higher percentage of the total plasma Zinc as albumin bound.

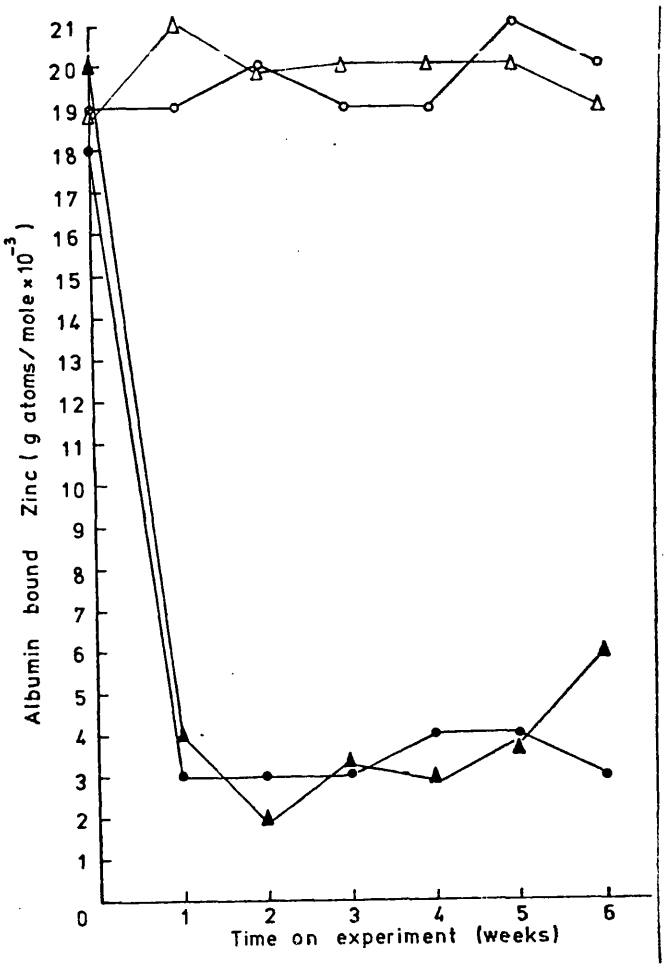


FIG. 19 EFFECT OF DEFICIENCY ON ALBUMIN BOUND ZINC IN PLASMA

●—●—● Zinc deficient male

○—○—○ Ad lib male control

▲—▲—▲ Zinc deficient female

△—△—△ Ad lib female control

TABLE 34

The Amount of Albumin Bound Zinc (g.atoms/mole) in Male and Female Lambs (Molecular Weight of Albumin 60,500)

Weeks	Male Lambs		Female Lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	0.018	0.019	0.020	0.019
1	0.003	0.019	0.004	0.021
2	0.003	0.020	0.002	0.020
3	0.003	0.019	0.003	0.020
4	0.004	0.019	0.003	0.020
5	0.004	0.021	0.004	0.020
6	0.003	0.020	0.006	0.019

TABLE 35

The Amount of Albumin Bound Zinc
as a Percentage of the Total Plasma Zinc

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	60.3	66.0	65.5	63.5
1	24.4	61.6	34.3	66.0
2	34.5	60,4	26.7	64.4
3	33.3	59.5	26.7	64.6
4	37.0	60.4	32.3	64.0
5	38.5	61.0	43.0	65.0
6	36.4	61.3	55.6	64.0

TABLE 36

Total Plasma Zinc (ug/ml)
for male and female lambs

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	1.15	1.14	1.16	1.15
1	0.40	1.12	0.35	1.17
2	0.29	1.08	0.30	1.15
3	0.30	1.10	0.30	1.16
4	0.27	1.10	0.30	1.16
5	0.26	1.12	0.28	1.17
6	0.23	1.07	0.27	1.17

α2-macroglobulin Bound Zinc

Table 37 and Figure 20 show that the α2-macroglobulin in Zinc deficient lambs retained bound Zinc at comparable levels to α2-macroglobulin bound Zinc in controls for two to four weeks after first feeding the Zinc deficient diet. Eventually in both the Zinc deficient males and females, the α2-macroglobulin bound Zinc decreased significantly ($P < 0.01$) by week 6. The males and females differed in the length of time that the α2-macroglobulin retained bound Zinc.

In the Zinc deficient males, the α2-macroglobulin retained bound Zinc for two weeks. This bound Zinc decreased significantly ($P < 0.01$) in week 3 from 1.26 g.atoms to 0.34 g.atoms Zinc/mole α2-macroglobulin in week 6. The control group retained a relatively constant amount of α2-macroglobulin bound Zinc during the entire six weeks.

In the Zinc deficient females, however, the α2-macroglobulin retained bound Zinc for four weeks. The α2-macroglobulin bound Zinc decreased significantly ($P < 0.01$) in week 5 from 0.84 g.atoms to 0.19 g.atoms Zinc/mole α2-macroglobulin in week 6.

Table 38 shows that the proportion of the total plasma Zinc bound by α2-macroglobulin in the Zinc deficient groups increased during the experimental period. The highest percentage of bound Zinc in the deficient males was 68.3 per cent in week 1, and in Zinc deficient females was 66.7 per cent in week 2.

It was noticeable, however, from the results that this proportion of bound Zinc showed some decrease before

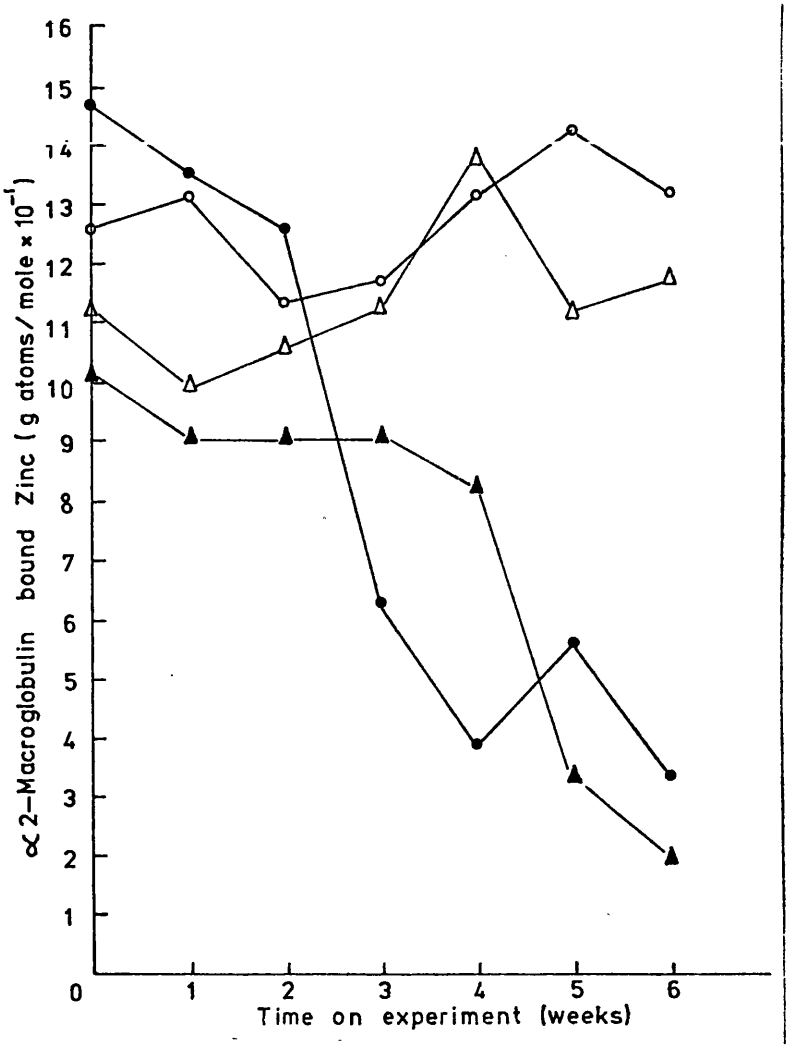


FIG. 20 EFFECT OF ZINC DEFICIENCY ON α2-MACROGLOBULIN BOUND ZINC IN PLASMA

●—●—● Zinc deficient male

○—○—○ Ad lib male control

▲—▲—▲ Zinc deficient female

△—△—△ Ad lib female control

TABLE 37

The Amount of α2-macroglobulin Bound Zinc (g.atoms/mole) in Male and Female Lambs
(Molecular weight of 2-macroglobulin 790,000)

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	1.483 NS	1.264	1.009 NS	1.118
1	1.361 NS	1.312	0.912 NS	0.997
2	1.264 NS	1.130	0.899 NS	1.069
3	0.644 *	1.167	0.899 NS	1.118
4	0.389 *	1.313	0.839 NS	1.373
5	0.583 *	1.422	0.340 *	1.118
6	0.340 *	1.313	0.194 *	1.167

Compared with control of same sex

*P $\leq$ 0.0

NS - not significant

TABLE 38

The Amount of Zinc Bound by α2-macroglobulin as a Percentage of the Total Plasma Zinc

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	24.0	22.0	17.2	19.5
1	68.3	25.0	51.4	19.0
2	52.0	22.5	66.7	18.6
3	50.0	21.0	66.7	21.0
4	37.0	23.4	58.0	23.0
5	38.5	25.0	28.5	20.0
6	45.5	24.5	18.5	20.0

the end of the period, especially in the Zinc deficient female group where the percentage of the total plasma Zinc bound by α2-macroglobulin decreased to pair-fed control group values.

Unbound Plasma Zinc

Table 39 shows that the amount of unbound Zinc in lamb plasma decreased significantly ($P < 0.01$) during the first week of feeding a Zinc deficient diet from a mean of 19.0 µgZn. to 4.0 µgZn./100 ml. The low values for unbound Zinc persisted for the remaining five weeks of the experimental period.

Table 40 shows that in Zinc deficient lambs the percentage of the total plasma Zinc which was unbound did not decrease during the period. Instead there was a slight increase in the percentage of bound Zinc between the first and last weeks of the experimental period. In the Zinc deficient males, the percentage of unbound Zinc increased from 15 to 18 per cent over six weeks, whilst in the Zinc deficient females, the percentage of unbound Zinc increased from 17 to 26 per cent.

TABLE 39

The amount of Unbound Zinc (µg/100 ml) in Plasma of Zinc Deficient Lambs

Weeks	Male lambs		Female Lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	18.00 NS	14.00	20.00 NS	20.00
1	3.00 *	15.00	5.00 *	18.00
2	4.00 *	19.00	2.00 *	20.00
3	5.00 *	22.00	2.00 *	17.00
4	7.00 *	18.00	3.00 *	15.00
5	6.00 *	16.00	8.00 *	18.00
6	4.00 *	15.00	7.00 *	19.00

Compared with control of same sex

*P <0.01

NS - not significant

TABLE 40

The Amount of Unbound Zinc
as a Percentage of the Total Plasma Zinc

Weeks	Male lambs		Female lambs	
	Zinc deficient group	Pair fed control group	Zinc deficient group	Pair fed control group
0	15.5	12.0	17.0	17.0
1	7.3	13.0	14.3	15.0
2	14.0	17.0	7.0	17.0
3	17.0	20.0	7.0	15.0
4	26.0	16.0	10.0	13.0
5	23.0	14.0	28.0	15.0
6	18.0	14.0	26.0	16.0

CHAPTER 11

Discussion

The results in Chapter 5 showed that when lambs consumed a diet which was severely deficient in Zinc, their performance was poor. This was noticeable within the first two weeks of feeding the diet. Measurements of food intake, growth, and food conversion efficiency showed no apparent indication that the Zinc deficient lambs had been able to adapt to this deficiency during a six week experimental period. Moreover, the progressive increase in external clinical symptoms shown in Chapter 6 further showed the apparent inability of the lambs to adapt to the 98 per cent decrease in the Zinc level of the diet.

Although these results confirm those by Mills et al (1967) and Ott et al (1964) they do not confirm the suggestion by Miller (1969), Miller (1970) and Miller et al (1970) that ruminants have a homeostatic control mechanism for Zinc. These workers showed that homeostatic control of Zinc existed and functions mainly through increasing the absorption of Zinc from the gut. With low Zinc diets of 2 mgZn./Kg, up to 80 per cent increase in ^{65}Zn absorption was recorded, whilst with dietary Zinc level of 16.0 mgZn./Kg, only 53 per cent of ^{65}Zn was absorbed. They concluded from these results on calves that ruminants in general, possessed a similar homeostatic control of Zinc. It was further suggested by Miller (1969) that this homeostatic mechanism in ruminants, does not fail with a very severe Zinc deficiency.

As the Zinc deficient diet was fed ad libitum to the lambs in the present experiment, it would have been expected that the lambs increased their absorption of Zinc from the intestine by up to 80 per cent at least, if similar mechanisms were functioning in lambs as in calves. This did not take place. It would appear, therefore, from a consideration of the whole animal and its husbandry, that a diet containing 0.9 mgZn./Kg may have been too severely Zinc deficient thus causing the homeostatic mechanism to break down, or alternatively a homeostatic mechanism continued to function at a much lower level, sufficient to maintain essential life functions.

When the Zinc deficient lambs were subsequently fed Zinc supplemented diets, their performance improved very rapidly each week. This suggests that the tissue resources of Zinc could not have been completely depleted, and further suggests that Zinc must have continued to be in dynamic equilibrium not only within different tissues, but also between the tissues and plasma. If a homeostatic control mechanism for Zinc was situated in the plasma, this would be ideally located for distributing Zinc to all the tissues in the body, and thus maintain a dynamic equilibrium with tissue Zinc. Such a mechanism of control could function at minimal level of Zinc intake.

The effect of the Zinc deficient diet on the performance of lambs involved the food conversion, growth, and the food intake of the lambs.

Food conversion in the Zinc deficient lambs was three to four times less efficient than either the pair-fed

or the ad libitum control groups. When the Zinc deficient lambs were fed the Zinc supplemented diets containing 40 mgZn./Kg, a rapid improvement in food conversion was subsequently observed; this compared favourably with control values even within one week of supplementing the diet.

These results not only confirm the poor food conversion reported by Ott et al (1964) in Zinc deficient sheep, but they also confirm the rapid recovery in performance, especially in food conversion, within the first week of feeding a Zinc supplemented diet. The Zinc content of the deficient diets used by Ott et al was 2.7 mgZn./Kg; this was not as low as the Zinc content, 0.9 mgZn./Kg diet, used in the present experiments. Therefore it could be argued that a longer recovery time would have been expected to restore normal food conversion in lambs fed a supplemented diet containing 40 mgZn./kg and previously on 0.9 mgZn./Kg, than in lambs of Ott et al which were fed a supplemented diet of 100 mgZn./Kg and previously on a low Zinc diet of 2.7 mgZn./Kg. However, a longer recovery time did not occur.

The rapidly improved food conversion in the Zinc deficient lambs above, when treated with different concentrations of Zinc in the supplemented diets, leads to the suggestion that the plasma proteins may have had a central role in the proportion of the plasma Zinc distributed and made available for growth.

The results of the mean growth rate of the Zinc deficient lambs during the six week period was significantly

(P $<$0.001) lower than the control groups. However, this lowered growth rate was not significantly apparent until weeks 3-4. Whilst this result is in agreement with Ott et al (1964) who also found that Zinc deficiency did not affect growth rate significantly for the first three weeks, Mills et al (1967), in a similar experiment, found that growth was arrested during the first week of feeding a Zinc deficient diet. This latter result might have been explained by the lower Zinc content, 1.2 mgZn./Kg, of the diet used by Ott et al. In the present experiment the Zinc content of the diet was 0.9 mgZn./Kg, which was comparable to the Zinc content of diets used by Mills, nevertheless, the growth response was more comparable to results by Ott et al.

It could be argued that lambs had very limited capacity to store Zinc in a form that could be used for growth during periods of low dietary Zinc intake. The Clun Forest Lambs, used in the present experiments and the cross-bred native lambs used by Ott et al appeared on this basis of not responding quickly to lack of Zinc in the diet, to have slightly greater capacity to maintain growth and possibly Zinc retention in binding, than the Dorset Horn lambs used by Mills et al.

In the present experiments the response of the Zinc deficient lambs to the Zinc supplemented diets was shown by a rapid increase in growth rate. This increase occurred even in the first week of supplementation despite the fact that the lambs had been made deficient for as long as six weeks.

This phenomenon of rapid recovery indicates that the supplemental dietary Zinc was rapidly 'topping up' the tissue Zinc via the plasma. An efficient method of Zinc transport in the plasma would therefore be important in establishing a rapid tissue response. Little is known on how Zinc is carried in the plasma and the role of plasma proteins in the transport of this element.

The difference in growth response between the lambs in the present experiment and those used by Mills et al or Ott et al, may have been due to a genetic factor; this is suggested because different breeds of lambs were used in all three experiments. The Zinc content of the pre-experimental diets fed to these lambs may also have had an influence on their subsequent growth rates; if such Zinc contents however had been recorded, and if the dietary Zinc fed to lambs used by Mills et al, was low prior to feeding the experimental diet, then it is possible that such lambs might have been near a threshold of Zinc deficiency. They would have shown depressed growth rates more rapidly than lambs which had been fed pre-experimental diets with a higher Zinc content.

The effect of dietary Zinc deficiency on the intake of food by lambs was shown in Chapter 5. During the experimental period of six weeks, lambs consumed a significantly ($P < 0.001$) lower amount of Zinc deficient diet (0.9 mgZn./Kg) than the lambs in the control group (40.0 mgZn./Kg diet). The results showed that even in the first week of the experiment, lack of Zinc in the diet decreased the intake significantly ($P < 0.01$)

Several workers including Miller et al (1965), Mills and Quarterman (1969), and Miller (1970) have previously reported that experimentally induced Zinc deficient animals consume less food than control animals fed supplemented diets. Their results suggest strongly however that this decrease in food intake was not simply due to a difference in taste between the two types of diet.

Although the basis control of voluntary food intake in ruminants is not fully understood, it is nevertheless accepted that a central hypothalamic mechanism is involved. This has been described by Hammond (1971). Studies by Tarttelin and Bell (1968) have shown that the hypothalamic centre in sheep is divisible into two reciprocally innervated centres, the feeding centre and the satiety centre. The latter centre is dominant and capable of overriding the feeding centre. This leads to the possibility that the blood supply to these centres could carry metabolites or even Zinc ions which might influence the activity of the centres directly. More research is needed along this line of investigation, especially as applied to food intakes of farm animals. It is interesting to note, however, that Mills (1970) suggested that reduced food intake in Zinc deficiency might be due to a metabolite produced in response to the deficiency and that the food intake continued to be suppressed until this metabolite was cleared. He makes no further suggestions as to how the metabolite could function. It could have been suggested, however, that this metabolite stimulated the satiety centre, which inhibited the feeding centre and in turn therefore caused a decrease in food intake.

The food intake of the Zinc deficient lambs did not show any change in the first or subsequent weeks when fed the Zinc supplemented diet. It is proposed therefore, in the light of the above suggestion, that the satiety centre continued to be stimulated because insufficient time had elapsed to clear the metabolite mentioned by Mills. Bell (1971) reported that the biochemical activity of the hypothalamus was difficult to study during satiety or feeding. His results suggested that more than a single metabolite controls the activity of the neural regulating process in feeding.

Throughout the studies on performance of lambs fed Zinc deficient diets, there was minimal difference between the results for male and female lambs.

The effect of Zinc deficiency on plasma alkaline phosphatase activity was shown by a gradual decrease over six weeks until a low mean enzyme activity of 2.04 Sigma units was obtained after six weeks of deficiency. A similar decrease in enzyme activity was also obtained in response to dietary Zinc deficiency in Period 2, but the low enzyme activity of 1.09 Sigma units was achieved in only three weeks instead of the six weeks taken during Period 1. In both these instances the time taken for the enzyme to decrease its activity was longer than for the plasma Zinc level to decrease in response to the Zinc deficient diet. Alkaline phosphatase molecules have been shown to contain Zinc by Mathies (1958), Engström (1961), Levinthal and Vallee (1962) and the enzyme activity was dependent on Zinc. Thus when Zinc was deficient in the plasma of lambs

in the present experiment, it was expected that alkaline phosphatase activity would show a rapid decrease. This expectation was not fulfilled as shown by results in Chapter 6; because sufficient Zinc was probably available in quantities suitable to maintain alkaline phosphatase activity.

A decrease in the plasma alkaline phosphatase activity in response to dietary Zinc deficiency has also been reported by Luecke et al (1957) and Oberlas et al (1966) in pigs, and by Miller et al (1965) in calves. No similar work has previously been reported for lambs. The present results also confirm the other results reported in that plasma alkaline phosphatase activity took much longer than plasma Zinc to respond to dietary Zinc deficiency.

A difference in activity of alkaline phosphatase occurred between male and female lambs fed Zinc deficient diets. The Zinc deficient females had significantly ($P < 0.001$) lower enzyme activity values than male lambs. This result was in contrast to results by Miller et al (1970) who found that Zinc deficient male pigs had significantly ($P < 0.01$) lower plasma alkaline phosphatase activity values than females. In both the present results and Miller's, although the sexes differed in the alkaline phosphatase results when fed Zinc deficient diets, the total plasma Zinc concentration remained at similar low levels in the male and female lambs. It could be argued that in Zinc deficient lambs, the total plasma Zinc is not a very direct measure of the Zinc available for physiological activity as Kirchgessner and Schwarz (1975) have suggested from their results with lactating cows; their evidence

indicated that serum alkaline phosphatase activity is a more reliable guide in diagnosing Zinc deficiency than total serum Zinc.

There is a need emerging from these results for further investigations along several possible lines; whether all plasma Zinc in lambs is totally available for physiological activity, whether plasma Zinc is divided into available and unavailable or bound fractions, or whether in normal animals Zinc is present at concentrations much higher than that actually needed for full physiological functions.

External clinical symptoms of Zinc deficiency were recorded as shown in Chapter 6 for six weeks. When these results were compared with the corresponding plasma alkaline phosphatase activity during the same period, it was seen that the trend of decreasing enzyme activity was similar to the trend of gradual deteriorating appearance of the lambs. An interesting symptom seen on the skin of lambs after the wool had been shed was the pin prick sized haemorrhages which appeared after three weeks of deficiency. A possible explanation for this stems from observations by Parry and Lacey (1975) who found that the blood clotting process in Zinc deficient lambs decreased during six weeks; their results showed that this was probably due to the significant ($P < 0.01$) reductions in the levels of clotting factors V and X.

These haemorrhages occurred during the same week that plasma alkaline phosphatase activity started to decrease significantly ($P < 0.05$). Although this observation has not been reported previously in lambs, a similar symptom was seen in Zinc deficient calves by Miller et al (1965). He

observed a close relationship between the severity of the skin lesions and plasma alkaline phosphatase activity. This close relationship was not seen in the present experiments to the same extent.

It might be argued that the skin lesions became apparent after a possible homeostatic mechanism for plasma Zinc control had broken down during the severe Zinc deficiency. Therefore, if the Zinc deficiency had been less severe and for a shorter period, then homeostatic control for Zinc in the plasma might have maintained a sufficient concentration of physiologically available Zinc to avoid the appearance of skin haemorrhages.

One of the main aims of this thesis was to investigate whether Zinc deficient lambs could adapt to maintain plasma protein levels. Previous experimental evidence has shown a relationship between Zinc and proteins. This has been observed, not only in the Zinc binding properties of human plasma proteins shown by several workers including Vikbladh (1951), Wolff (1956) and Giroux (1975), but also a Zinc protein relationship has been shown by Mills et al (1969) who showed that Zinc was necessary for protein synthesis in rats, and by Van Campen and House (1973) who showed that low dietary protein was correlated with low plasma Zinc.

The results in Chapter 7 showed that the mean plasma protein concentration increased signficantly ($P < 0.01$) each week in lambs fed Zinc deficient diets, whilst lambs fed the Zinc supplemented diets maintained a relatively constant concentration of plasma proteins throughout.

Results of similar studies by Ott et al (1964)

also showed a consistent increase in plasma protein concentration during Zinc deficiency in lambs, but this increase was not so large as in the present experiment, and also it did not reach statistical levels of significance. Similarly, the consistent increased concentration of plasma proteins in experiments reported by Miller et al in Zinc deficient pigs was less than in experiments reported by Ott et al. If the size of these three protein responses to Zinc deficiency are compared with the Zinc content of the respective diets:-

Zinc content of present diet: 0.9 mgZn./kg.
" " " Ott's diet: 2.7 mgZn./Kg.
" " " Miller's diet: 12.0 mgZn./Kg.

then it is noticeable that the greatest increase in plasma protein concentration occurred with the lowest dietary Zinc, whilst the least increase in protein concentration occurred with the highest dietary Zinc.

The response of the Zinc deficient lambs to Zinc supplementation was immediate, with the raised concentration of plasma proteins decreasing until normally accepted control levels of plasma proteins resulted after six weeks.

The concentration of albumin and globulin fractions were measured and recorded in Chapter 7. The results showed that the mean globulin concentration of the Zinc deficient lambs had increased significantly ($P < 0.01$) in concentration each week, and it was significantly ($P < 0.01$) higher during the six week experiment than the globulin concentration of the Zinc supplemented control group.

In contrast to the globulin results, the albumin concentration of the Zinc deficient lambs was significantly

($P < 0.001$) lower than the Zinc supplemented lambs, and decreased gradually but significantly ($P < 0.01$) each week.

When Zinc supplemented diets were fed, however, both the albumin and globulin levels in the Zinc deficient lambs showed an immediate response by increasing and decreasing in concentration respectively. These changes in both proteins were significant ($P < 0.01$) each week until their concentrations had returned, after five weeks, to similar concentrations found in control lambs. This evidence indicates that Zinc stimulated the synthesis of albumin, whilst it inhibited the synthesis of globulins. This would therefore suggest that the concentration of albumin was low during Zinc deficiency because of lack of stimulus for its synthesis.

The trend of higher globulin and lower albumin concentrations shown above in Zinc deficient lambs agrees with previously reported results by Ott et al (1964) in lambs, by Hoefer et al (1960), Smith et al (1960) and Miller et al (1968) in pigs, and by Miale et al (1963) in human patients. The increased globulin concentration also confirms the increased fibrinogen level found by Parry and Lacey (1975) in Zinc deficient lambs. Their results showed that the fibrinogen level increased consistently during six weeks.

Results by Ott et al showed that whilst the globulin increased significantly ($P < 0.01$), no significant lowering of albumin concentration was observed. Results in pigs, reported by Miller et al (1968), showed significant increases and decreases in globulin and albumin fractions respectively, and are thus in agreement with the results

for lambs in the present experiments.

The results of estimating the individual globulin fractions, viz: α_1-, α_2-, β-, and γ-globulins in Zinc deficient lambs, showed that significant increases in concentration occurred in these fractions during the experimental period; whilst the concentration in the control lambs remained constant. It is interesting to note the percentage increase in concentration of globulin fractions after six weeks of Zinc deficiency, as set out below:-

	Period 1	Period 2
α_1-globulin	396	338
α_2-globulin	48	112
β-globulin	167	146
γ-globulin	139	144
Total-globulin	158	160

Whilst the β- and γ-globulin fractions increased by similar percentage rises as the total globulin, the α_1 and α_2-globulin fractions were affected differently by the Zinc deficiency. The α_1-globulin showed the highest percentage rise, whilst α_2-globulin showed the least percentage rise during both deficiency periods. It is interesting to note that the α_2-globulin fractions contains α_2-macroglobulin, which was shown in Chapter 10 to be the Zinc containing protein showing least change in Zinc content during deficiency.

The response of the Zinc deficient lambs to the Zinc supplemented diet, was again immediate, with the concentrations of globulin fractions decreasing during six weeks until their concentrations had returned to control levels.

Ott et al (1964) also found that the γ-globulin concentration increased significantly in Zinc deficient lambs, but they did not estimate separately the α- and β-globulin fractions. Miller et al (1968) has also found a similar increase in α2- and γ-globulin concentration in Zinc deficient pigs; their α1-globulin, however, decreased whilst the β-globulin concentration was the same in both Zinc deficient and Zinc supplemented pigs.

The β- and γ-globulin fractions showed no differences in concentration between male and female lambs in Zinc deficiency. The α1- and α2-globulins, however, had higher concentrations in the males than in the females. It is tempting to speculate from these results that the female had a more efficient mechanism of homeostatic regulation of plasma Zinc levels and hence the regulation of α1- and α2-globulins. Evidence which goes somewhat to support this speculation has come from work by Yunice et al (1975). They found that a balance could be operating in the female between oestrogens causing a decrease, and progesterone causing an increase in plasma Zinc; thus the female might be more protected than males against the effects of Zinc deficiency. No previous reports have been found published on differences between Zinc deficient male and female lambs.

It would seem probable that the increased total protein concentration reported earlier, was due to the increased concentration of the globulin fraction. Ott et al (1964) suggested that the increased globulin concentration was probably a response to secondary infection from the open lesions on the skin. This does not seem to be a

possible explanation from the results of the present experiment because the globulin concentration increased significantly ($P<0.01$) even during the first and second weeks of feeding the Zinc deficient diet, before the skin lesions became apparent in the third week.

Throughout these studies there was evidence that the metabolism of plasma proteins changed in lambs when they were fed Zinc deficient diets. The fact that the globulin concentration increased and albumin concentration decreased, suggests a diminution in the ability to utilize the globulins, and possibly a diminution in the lamb's ability to synthesize albumin. This suggestion becomes more probable when evidence linking Zinc with protein synthesis or protein breakdown or even with both of these processes, is considered.

A role for Zinc in protein synthesis has been suggested from studies on micro-organisms by Nason et al (1951), Winder and Dennery (1959), and Wacker (1962). Later, several workers, Fujioka and Liebenman (1964), Williams, Mills, Quarterman and Dalgarno (1965), and Williams and Chesters (1970), investigated the effect of Zinc deficiency on rates of synthesis of protein and DNA within mammalian tissues. There was general agreement from their results that Zinc deficiency caused more marked inhibition of DNA synthesis than of protein synthesis. Later, Slater, Mildran and Laeb (1971) reported that Zinc was a constituent of DNA polymerase. Therefore it is possible that Zinc is a requirement for the synthesis of certain enzymes involved in DNA synthesis.

Although evidence is available to indicate the involvement of Zinc in protein synthesis, it does not give a clear explanation for the increased globulin concentration when Zinc is deficient. It is nevertheless possible that a deficiency of Zinc caused a decrease in the synthesis of an enzyme involved in the metabolism of plasma globulin. The breakdown of globulins would therefore decrease with the consequence that their concentration in plasma would increase.

Accepting, however, that Zinc is involved in protein synthesis, then the question of how this mechanism is sensitive to changes in dietary Zinc levels remains unanswered. When the lambs were given the Zinc deficient diet, although the total plasma Zinc concentration decreased rapidly in one week, the growth rate and plasma proteins did not decrease as rapidly. This indicates that a homeostatic mechanism was probably trying to maintain a level of Zinc in a form suitable for physiological functions, such as protein synthesis. Little information is available in the literature on the proportion of bound and unbound Zinc in lamb plasma.

The results in Chapter 8 of investigating the proportion of unbound and protein bound Zinc in normal lamb plasma showed that 93 per cent of total plasma Zinc was bound to proteins; 7 per cent was calculated to be unbound. The method used to determine these two proportions of Zinc depended on the retention of protein molecules by a membrane containing a pore size which was too small for the passage of molecules with molecular weights greater than 500. The molecules of Zinc which were not bound to proteins

of molecular weight greater than 500 would have passed through the membrane and become the unbound Zinc fraction. This fraction probably also contained amino acids, because most of them would have molecular weights less than 500. Therefore it could be argued that possibly not all the 7 per cent unbound Zinc was physiologically available because some was bound to amino acids, as suggested by Prasad and Oberleas (1970).

Although no report has been found giving an estimate of unbound Zinc in sheep plasma, in human plasma however, Prasad and Oberleas (1968) showed that no more than 3 per cent was unbound, and 97 per cent was protein bound. Earlier reports however by Vikbladh (1951), Underwood (1962), and Prasad (1966) suggested that all Zinc in human plasma was protein bound. These latter reports however made no distinction between weakly and strongly bound Zinc, and made no suggestions on how the bound Zinc would be affected in Zinc deficient conditions.

In the light of these results it was considered reasonable to estimate unbound plasma Zinc in lambs to be between 3 and 7 per cent of the total plasma Zinc.

The effect of dietary Zinc deficiency on the unbound and protein bound Zinc in lamb plasma was shown in Chapter 8, Figure 15. These results showed that the concentration of unbound Zinc in the Zinc deficient lambs during the first week remained unchanged at a mean value of 0.1 µg/ml despite the fact that the total plasma Zinc concentration decreased by 65.5 per cent from 1.16 to 0.40 µg/ml. The total plasma Zinc decreased even further during the six week period without making any significant change in the concentration of unbound Zinc.

A comparison between these results for unbound plasma Zinc and the activity of alkaline phosphatase in Zinc deficient lambs seen earlier in Figure 7, indicates that although the concentration of unbound Zinc remained unchanged, the enzyme activity showed a gradual decrease. No sudden decrease in activity occurred in total plasma Zinc. The total plasma Zinc and total bound Zinc started to decrease at the same time and almost at similar rates.

These results suggest that unbound Zinc was being made available at the expense of the bound Zinc; in this way a constant level of unbound Zinc was maintained throughout. It is difficult to account for the continuing decrease in alkaline phosphatase activity whilst the unbound Zinc level was held constant, unless some of the estimated unbound Zinc was actually bound to amino acids as suggested by Prasad and Oberleas (1970) therefore being unavailable for physiological functions. It was apparent that further investigations were needed to locate the Zinc binding plasma proteins.

The results in Chapter 9 show that Zinc was bound to two plasma proteins - albumin and α_2-macroglobulin, in Zinc supplemented lambs. No previous reports have been found describing the location of bound Zinc in plasma of lambs, or ruminant plasma. In human plasma, however, Parisi and Vallee (1970), and Boyett and Sullivan (1970), have previously separated both albumin and α_2-macroglobulin as the two Zinc binding proteins. The quantity of Zinc bound by albumin and α_2-macroglobulin was determined in both the Zinc deficient and Zinc supplemented control lambs.

In the Zinc supplemented control lambs the percentage of total plasma Zinc bound to albumin was 63 per cent, and to α2-macroglobulin was 22 per cent. These proportions of bound Zinc in lambs were similar to results in human plasma by Parisi and Vallee (1970) in as much as they showed more Zinc bound to albumin than α2-macroglobulin. They found 60 per cent of total Zinc to be albumin bound and between 30-40 per cent of α2-macroglobulin bound Zinc. Boyett and Sullivan (1970) found that up to 80 per cent of total human plasma Zinc was albumin bound and only 15 per cent was α2-macroglobulin bound Zinc. More recently Girauz and Henkin (1972) found that 85-90 per cent was albumin bound.

The effect of dietary Zinc deficiency (reported in Chapter 10) was shown to influence greatly the proportion of total plasma Zinc which was bound to albumin and to α2-macroglobulin. The percentage of albumin bound Zinc decreased to approximately half compared with Zinc supplemented lambs, whilst the percentage of α2-macroglobulin bound Zinc more than doubled compared with Zinc supplemented lambs.

This result is important because it throws light on a possible homeostatic mechanism which controlled the bound and unbound plasma Zinc. Previous reports by Wolff (1956), Prasad (1966), Parisi and Vallee (1970) and Widdowson (1974), have established that plasma Zinc was loosely bound to albumin in such a way that it was more easily exchangeable than the more firmly bound α2-macroglobulin bound Zinc. Most of these previous studies used isotopic Zinc and in vitro plasma, to establish that a large percentage of Zinc was loosely bound. Results from the present experiments

have extended these previous studies in two directions. Firstly, they have defined the effect of Zinc deficiency on the proportion of Zinc bound to albumin and α2-macroglobulin in lamb plasma rather than an in vitro control study, and secondly, ruminant plasma has been studied rather than human plasma.

Before the importance of the above mentioned result can be appreciated fully in the context of a possible homeostatic mechanism in the plasma for controlling unbound Zinc levels in Zinc deficiency, the results of the concentration and Zinc binding capacity of each binding protein must be considered in more detail.

The albumin concentration of the Zinc deficient lambs, decreased gradually during the six week experimental period shown in Chapter 10, Table 30. In the first week both the total plasma Zinc and the percentage of the total Zinc which was albumin bound, decreased rapidly. This decrease was due to a reduction in the amount of Zinc per mole of albumin, Figure 19, Table 34; this showed a good correlation with the total plasma Zinc levels. This result therefore suggested that there were less binding sites on the albumin molecule capable of binding Zinc during Zinc deficiency rather than less binding sites because of decreased amount of albumin. This showed that Zinc must have been loosely bound to the albumin, thus confirming in lambs similar results found previously for human plasma by Wolff (1956), Prasad (1966), Parisi and Vallee (1970) and Widdowson (1974).

The α2-macroglobulin concentration of the Zinc deficient lambs increased gradually during the six week period. In the first week of deficiency, the percentage

of the total plasma Zinc which was bound to α2-macroglobulin increased rapidly. However, this apparent increase was due to the α2-macroglobulin binding the same amount of Zinc per mole of protein, but from a decreasing total quantity of plasma Zinc; thus the percentage was inevitably higher as the total plasma Zinc decreased.

It was noticeable that the α2-macroglobulin in the Zinc deficient female lambs maintained a constant amount of bound Zinc for four weeks before its binding capacity decreased rapidly in the fifth week of the experiment. The Zinc deficient males however were different, and only maintained bound Zinc to α2-macroglobulin for the first two weeks of the experiment. This however may be another example which demonstrates the greater protection against Zinc deficiency in females, mentioned earlier, afforded by the balance between oestrogens and progesterone suggested by Yunice et al (1975).

All these results showed that dietary Zinc deficiency had an effect on Zinc binding properties of albumin and α2-macroglobulin. Zinc was loosely bound to albumin because less Zinc was bound per mole albumin, even as early as during the first week of Zinc deficiency. The Zinc bound to α2-macroglobulin was more tightly bound because the binding sites on the protein retained Zinc for a longer period than in albumin. This suggests therefore, that α2-macroglobulin was part of a homeostatic mechanism to retain Zinc in the plasma. After three to four weeks of Zinc deficiency, however, the results showed that Zinc binding per mole of α2-macroglobulin decreased. After

this relatively prolonged period of deficiency, the homeostatic function of this protein may have broken down.

A comparison of Tables 30 and 37 shows that the concentration of α2-macroglobulin was increasing during the same time (weeks 4, 5, and 6) as fewer binding sites for Zinc on the protein molecule were available. This suggests therefore, that after prolonged Zinc deficiency the amino acid composition or the conformation of the newly synthesized α2-macroglobulin molecules were different from the previously synthesized molecules. Alternatively the reduced availability of plasma Zinc may have fallen below the concentration for maximum binding by α2-macroglobulin.

The unbound Zinc has been expressed in Chapter 10 Tables 39 and 40. The suggestion that not all this Zinc is physiologically available but instead may be bound to amino acids, has already been discussed. It is however interesting to note that the decreased concentration of unbound Zinc occurred during the first week of deficiency at the same time as the decreased binding of Zinc to albumin.

In the light of these results, coupled with the fact that Zinc was loosely bound to albumin, it can be suggested that albumin was making unbound Zinc available in an attempt to maintain a relatively even proportion of total plasma Zinc for physiological needs.

Further evidence to support this suggestion can be drawn from the results in Chapter 8 which showed the percentage of total plasma unbound Zinc determined by ultrafiltration. This percentage was considerably higher during the first four to five weeks of Zinc deficiency than in the Zinc supplemented plasma.

It could be argued therefore, that Zinc deficiency was a direct or indirect cause of Zinc being dissociated from albumin. The resulting unbound Zinc, in whole or in part, might have become bound to low molecular weight peptides or amino acids and passed through the pores of the ultrafilter membrane and thus contributed to the unbound Zinc fraction.

From the behaviour of albumin in experimental Zinc deficient lambs, the results suggest that it has a role as part of a homeostatic mechanism which makes Zinc available rapidly. It would seem that Zinc is more loosely bound to albumin than it is to α2-macroglobulin and therefore albumin would release Zinc from its bound form and maintain the amount of unbound and hence physiological available Zinc.

Evidence has been presented to suggest that α2-macroglobulin retains its Zinc longer than albumin, and therefore had the role of a transport protein for Zinc in plasma. This retention persisted long after the total plasma Zinc had been reduced and after the equilibrium was upset. This property of α2-macroglobulin may be part of a mechanism which functions in severe situations.

These studies, whilst they have not fully clarified the homeostatic mechanism for regulating either bound or unbound plasma Zinc, have shown a relationship which has not previously been identified between total plasma Zinc, plasma protein bound and unbound Zinc in lamb plasma. Evidence has also been shown for suggesting a homeostatic control of Zinc in plasma.

A further suggestion is that the imposed Zinc deficiency in the present experiments was probably too severe to detect the finer points of homeostatic control between bound and unbound plasma Zinc and to correlate these biochemical symptoms with the more clinical and external symptoms of deficiency.

Therefore, further experiments are recommended in which lambs would be made marginally Zinc deficient so that homeostatic mechanisms could be studied before more severe deficiency caused a breakdown in such mechanisms. Results from these experiments would help in explaining how plasma Zinc levels can be maintained as well as the limits within which the homeostatic mechanisms can operate with dietary Zinc deficiency.

REFERENCES

Aitken, I., Lindsay, K.A., Hart, F., and McKay, J. (1973), Clin. Sci. 44 (1), 91.

Andersson, L.O. (1966), Biochim. Biophys. Acta. 117, 115

Andrews, P. (1964), Biochem. J. 96, 595.

Arora, S.P., Hatfield, E.D., and Garrigus, U.S. (1968a), J. Anim. Sci. 27, 1766.

Bell, F.R. (1971), Proc. Nutr. Soc. 30, 103.

Bertrand, G., and Bhattacherjee, R.C. (1934), Compt. r. Acad. Sci. 198, 1823.

Blackmon, D.M., Miller, W.J., and Morton, J.D. (1967), Vet. Med., 62, 265.

Borg, D.C., and Cotzias, G.C. (1958), J. Clin. Invest. 37, 1269.

Boyett, J.D. and Sullivan, J.F. (1970), Metabolism. 19, 148.

Buchanan, P.J. and Hsu, J.M. (1968), Fed. Proc. 27, 483.

Cassens, R.G., Briskey, E.J., and Hoekstra, W.G. (1963), J. Sci. Fd. Agri. 14, 427.

Cotzias, G.C., Borg, D.C., Selleck, B. (1962), Amer. J. Physiol. 202, 359.

Cotzias, G.C., Borg, D.C., and Selleck, B. (1961), Amer. J. Physiol. 201, 63.

Cotzias, G.C., and Papavasiliou, P.S., (1963), Amer. J. Physiol. 206, 787.

Davis, B.J. (1964), Ann. N.Y. Acad. Sci. 121, (2), 404.

Davies, D.G., and Baker, M.H. (1974), Vet. Rec. 94, 561.

Day, G.H., and McCollum P. (1940), Proc. Soc. Exp. Biol. Med. 45, 282.

Dukes, H.H. (1955), The Physiology of Domestic Animals, p. 668, 7th ed. Ithaca: Comstock Publishing Assoc.

Duncan, D.B. (1955), Biometrics. 11, 1.

Edsall, J.T., Felsenfeld, G., Goodman, D.S., and Gurd, F.R.N. (1954a), J. Am. Chem. Soc. 76, 3054.

Edsall, J.T., Maybury, R.H., Simpson, R.B., and Straessle, R. (1954b), J. Am. Chem. Soc. 76, 3131.

Engström, L. (1961), Biochim. biophys. Acta. 52, 36.

Evans, G.W., and Winter, T.W. (1975), Biochem. Biophys. Res. Comm. 66, 4, 1218.

FAO/WHO (1974), Anim. Hlth. Yb. 1974.

Flynn, A., Pories, W.J., Strain, W.H., and Weiland, J. (1973), Naturwissenschapter, 60 (3), 162.

Fox, M.R.S., and Harrison, B.N. (1965), J. Nutr. 86, 89.

Fujioka, M., and Lieberman, I. (1964), J. Biol. Chem. 239, 1164.

Giroux, E.L. (1975), Biochemical Medicine12, 258.

Giroux, E.L., and Henkin, R.I. (1972), Biochim. Biophys. Acta, 273, 64.

Grashuis, J. (1964), Bemesting, Voeding, Gezondheid en Ziekte, Herziene druk. Arnhem: Landbouwk. Bureau Sporenelementen.

Gurd, F.R.N., and Goodman, D.S. (1952), J. Am. Chem. Soc. 74, 670.

Gurd, F.R.N., and Murray, G.R., Jr. (1954), J. Am. Chem. Soc. 76, 187.

Haaranen, S. (1963), Nord. Vet. Med. 15, 536.

Halstead, J.A., Hackley, B.M., and Smith, J.C. (1968), Lancet 2, 278.

Halstead, J.A. and Smith, J.C. (1970), Lancet i, 322.

Hambridge, K.M. (1974), In trace element metabolism in animals 2, Proc. 2nd Int. Synp. on Trace Element Metabolism in Animals p. 171. (W.G. Hoekstra, Editor) Butterworths London.

Hammond, J. Jr. (1971), Hammond's Farm Animals, 4th ed. London, p. 26.

Haverback, B.J., Dyce, B., Bundy, H.F., Wirtschafter, S.K., and Edmondson, H.A. (1962), J. Clin. Inv. 41, 972.

Henry, R.J., (1974) Clinical Chemistry, Principles and Technics. 3nd ed. Harper and Row.

Himmelhoch, S.R., Sober, H.A., Vallee, B.L., Peterson, E.A., and Fuwa, K. (1966), Biochemistry, 5, 2523.

Hoefer, J.A., Miller, E.R., Ullney, D.E., Ritchie, H.D., and Luecke, R.W. (1960), J. Anim. Sci. 19, 249.

Holod, V.M., Spak, G.E., and Guthovic, J.L. (1969), Nutr. Abstr. Rev. 39, 976.

Hove, E., Elvehjem, C.A. and Hart, E.B. (1940), Amer. J. Physiol. 119, 768.

Humphries, W.R., and Quarterman, J. (1968), Proc. Nutr. Soc. 27, 54A.

Hurley, L.S. and Swenerton, H. (1966), Proc. Soc. Exp. Biol. Med. 123, 692.

Hyppola, K. (1966), J. Sci. Agri. Soc. Finland 38, 180.

Kägi, J.H.R., and Vallee, B.L. (1960), J. Biol. Chem. 235, 3188.

Kernkamp, H.C.H., and Ferrin, E.F. (1953), J. Am. Vet. Med. A. 123, 217.

King, T.P., Yphantis, D.A., and Craig, L.C. (1960), J. Am. Chem. Soc. 82, 3350.

Kirchgessner, M., and Schwarz, W.A. (1975), Zentralbl. Veterinaermed., Reihe A. 22 (7), 572.

Kohn, J. (1958), Clin. Chim. Acta. 3, 450.

Koltun, W.L., Roth, R.H., and Gurd, F.R.N. (1963), J. Biol. Chem. 238, 124.

Legg, S.P., and Sears, L. (1960), Nature, Lond. 186, 1061.

Lindeman, R.D. et al. (1971), J. Gerontol. 26, 358.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951), J. Biol. Chem. 193, 265.

Luecke, R.W., Hoefer, J.A., Brammell, W.S., and Thorp, F. (1957), J. Anim. Sci. 15, 347.

Luecke, R.W., Olman, M.E., and Baltzer, B.V. (1968), J. Nutr. 94, 344.

Martin, R.B., and Edsall, J.T. (1960), J. Am. Chem. Soc. 82, 1107.

Mathies, J.C., (1958), J. Biol. Chem. 233, 1121.

Maynard, L.A., and Loosli, J.K. (1956), Animal Nutrition p.154, 4th ed. New York: McGraw Hill Book Co.

McBean, L.D., Smith, J.C., and Halsted, J.A. (1971), Proc. Soc. Exp. Biol. Med. 137, 543.

Mehl, J.W., O'Connell, W., and DeGroot, J. (1964) Science 145, 821.

Miale, A. Jr., Faris, Z., Sandstead, H.H., and Schulert, A.R. (1963), J. Lab. Clin. Med. 61, 537.

Miller, J.K. (1965), J. Dairy Sci. 48, 370.

Miller, W.J. (1969), Am. J. Clin. Nutr. 22, 1323.

Miller, W.J. (1970), J. Dairy Sci. 53, 1123.

Miller, W.J. (1971), In Mineral Studies with Isotopes in Domestic Animals, Vienna. Intern. Atomic Energy Agency, 23.

Miller, J.K., and Miller, W.J. (1960), J. Dairy Sci. 43, 1854.

Miller, J.K., and Miller, W.J. (1962), J. Nutr. 76, 467.

Miller, W.J., Blackmon, D.M., Gentry, R.P., and Pate, F.M. (1970), 31, 149.

Miller, W.J., Blackmon, D.M., Gentry, R.P., Pitts, W.J., and Powell, G.W. (1967), J. Nutr. 92, 71.

Miller, W.J., Blackmon, D.M., Gentry, R.P., Powell, G.W., and Perkins, H.F. (1966a), J. Dairy Sci. 49, 1446.

Miller, W.J., Blackmon, G.W., Powell, R.P., Gentry, R.P., and Hiers, J.M. (1966b), J. Nutr. 90, 335.

Miller, W.J., Clifton, C.M., Cameron, N.W. (1963), J. Dairy Sci. 46, 715.

Miller, J.K., and Cragle, R.G. (1965), J. Dairy Sci. 48, 370.

Miller, M.J., Fischer, M.I., Elcoate, P.V., and Mawson, C.A. (1958), Can. J. Biochem. Physiol. 36, 557.

Miller, W.J., Martin, Y.G., Gentry, R.P., and Blackmon, D.M. (1968a), J. Nutr. 94, 391.

Miller, J.K., Miller, W.J., and Clifton, C.M. (1962), J. Dairy Sci. 45, 1536.

Miller, W.J., Pitts, W.J., Clifton, C., and Schmittle, S.C. (1964), J. Dairy Sci. 47, 556.

Miller, W.J., Powell, G.W., Blackmon, D.M., and Gentry, R.P. (1968b), J. Dairy Sci. 51, 82.

Miller, W.J., Powell, G.W., and Hiers, J.M. (1966 c), J. Dairy Sci. 49, 1012.

Miller, W.J., Powell, G.W., Pitts, W.J. and Perkins, H.F. (1965c), J. Dairy Sci. 48, 1091.

Miller, W.J., Pitts, W.J., Clifton, C.M., and Morton, J.D. (1965), J. Dairy Sci. 48, 1329.

Miller, W.J., Pitts, W.H., Clifton, C.M., and Morton, J.D. (1965b), J. Dairy Sci. 48, 1329.

Miller, W.J., Pitts, W.J., Clifton, C.M., Schmittle, S.C. (1964), 47, 556.

Miller, W.J., Wells, E.S., Gentry, R.P., and Neathery, M.W. (1971), J. Nutr. 101, 1673.

Mills, C.F., (1974), Proc. Nutr. Soc. 33, 267.

Mills, C.F., and Chesters, J.K. (1970), In Trace Elements Metabolism in Animals, p.39 (C.F. Mills, Editor), Edinburgh: E. and S. Livingstone.

Mills, C.F., Dalgarno, A.C., Williams, R.B., and Quarterman, J. (1967), Brit. J. Nutr. 21, 751.

Mills, C.F., Quarterman, J., Chesters, J.K., Williams, R.B., and Dalgarno, A.C. (1969), Am. J. Clin. Nutr. 22, 1240.

Nason, D.W., Eaton, R.H., Smith, J.K., and Whitby, L.G. (1951), Biochem. J. 102, 53.

Neathery, M.W., Miller, W.J., Blackmon, D.M., Pate, F.M., and Gentry, R.P. (1973), J. Dairy Sci. 56 (1), 98.

Oberleas, D., Muherer, M.E., and O'Dell, B.L. (1966). In Zinc metabolism as the Availability of Zinc from Foodstuffs, p.225, (A.S. Prasad, Editor), Springfield, Ill: C.C. Thomas.

O'Dell, B.L., Newberne, P.M., and Savage, J.E. (1958), J. Nutr. 65, 503.

Ornstein, L., and Davies, (1964), Ann. N.Y. Acad. Sci. 121, (2), 321.

Ott, E.A., Smith, W.H., Stob, M. and Beeson, W.M. (1964), J. Nutr. 82, 41.

Ott, E.A., Smith, W.H., Stob, M., Parker, H.E., and Beeson, W.M. (1965), 24, 735.

Parry, W.H., and Lacey, J.T. (1975), I.R.C.S. Medical Science, 3, 523.

Parisi, A.F. and Vallee, B.L. (1970), Biochemistry. 9, 2421.

Perkins, D.J. (1961), Biochem. J. 80, 668.

Peterson, E.A., and Sober, H.A. (1956), J. Am. Chem. Soc. 78, 751.

Pierson, R.E. (1966), J. Amer. Vet. Med. Assoc. 149, 1279.

Pitts, W.J., Miller, W.J., Fosgate, O.T., Morton, J.D., and Clifton, C.M. (1966), J. Dairy Sci. 49, 995.

Plocke, D.J., Leventhal, C. and Vallee, B.L. (1962), Biochemistry, Easton 1, 373.

Pories, W.J., Henzel, J.H., Rob, C.G., and Strain, W.H. (1967), Lancet 1: 121.

Prasad, A.S. (1966), In Zinc Metabolism, p.250, Springfield, Ill: C.C. Thomas.

Prasad, A.S., and Oberleas, D. (1970), J. Lab. Clin. Med. 76 (3), 416.

Rahman, M.M., Davies, R.E., Deyoe, C.W., Reid, B.L. and Couch, J.R. (1961), Poultry Sci. 30, 195.

Raulin, J. (1869), Ann. Sci. Nat. Botan. Biol. reget. 11, 93.

Reider, H. (1959), J. Biol. Chem. 245, 160.

Sahagian, B.M., Harding-Barlow, I. and H.M. Parry (1967), J. Nutr. 93, 291.

Schönenberger, M., Schmidtberger, R., and Schultze, H.E. (1968), Z. Naturforsch, B. 13, 761.

Schultze, H.E., and Heremans, J.F. (1966), Molecular Biology of Human Proteins, Vol. 1; Amsterdam, Elsevier.

Semenkhanov, L. (1972), Chem. Abstracts. 82, 1441.

Shyy-Hwa, T., and Hurley, L.S. (1971), Proc. Soc. Exp. Biol. Med. 136, 165.

Shrivastava, P.K., Goch, H., and Zakrzewski, K. (1972) Biochim. Biophys. Acta. 271, 310.

Slater, J.P., Mildran, A.S., and Loeb, L.A. (1971), Biochem. Biophys. Res. Commun. 44, 37.

Smith, I. (1968), Chromatographic and Electrophonetic Techniques, 2nd ed. London: William Heinemann Ltd.

Smith, I.D., Grummer, R.H., Hoekstra, W.G., and Phillips, P.H. (1960), J. Anim. Sci. 19, 568.

Sober, H.A., Gutter, F.J., Wyckoff, M.M. and Peterson, E.A. (1956), J. Am. Chem. Soc. 78, 657.

Somers, M., and Underwood, E.J. (1969), Aust. J. Agr. Res. 20, 899.

Stake, P.E., Miller, W.J., and Gentry, R.P. (1972), Proc. Soc. Exp. Biol. Med. 142, 494.

Tarttelin, M.F., and Bell, F.R. (1968), J. Physiol. Lond. 195. 29P.

Todd, W.P., Elvehjem, C.A., and Hart, E.B. (1934), J. Amer. Physiol. 107, 146.

Tucker, H.F., and Salmon, W.D. (1955), Proc. Soc. Exp. Biol. Med. 88, 613.

Underwood, E.J. (1962), in Trace Elements in Animal and Human Nutrition, ed. 2, New York and London Academic Press.

Underwood, E.J., and Somers, M. (1969), Aust. J. Agric. Res. 20, 889.

Vallee, B.L. (1959), J. Am. Chem. Soc. 87, 4002.

Vallee, B.L. (1960), Physiol. Rev. 39, 443.

Vallee, B.L., and Neurath, H. (1954), J. Am. Chem. Soc. 76, 5006.

Vallee, B.L., and Neurath, H. (1955), J. Biol. Chem. 217, 253.

Vallee, B.L. and Wacker, W.E.C. (1970), In the Proteins as Metal-Binding Groups of Proteins, p.61 (H. Neurath, Editor) Vol. 5 London: Academic Press.

Vallee, B.L., and Williams, R.J.P. (1968B), Proc. Natl. Acad. Sci. U.S. 59, 498.

Vallee, B.L., and Williams, R.J.P. (1968a), Chem. Brit. 4, 397.

Van Campen, D. and House, W.A. (1973), J. Nutr. 104, 84.

Vessel, E.S., and Bearn, A.G. (1957), Proc. Soc. Exp. Biol. Med. 94, 96.

Vikbladh, I. (1951), Scand. J. Clin. Lab. Invest. 3, Suppl. 2.

Wacker, W.E.C. (1962), Biochemistry, Easton, 1, 859.

Widdowson, E.M., Dauncey, J., and Shaw, J.C.L., (1974), Proc. Nutr. Soc., 33, 275.

Williams, R.B., and Chesters, J.K. (1970), Br. J. Nutr. 24, 1053.

Williams, R.B., Mills, C.F., Quarterman, J., and Dalgarno, A.C. (1965), Biochem. J. 95, 29P.

Winder, F.G., and Denneny, J.M. (1959), Nature (London) 184, 742.

Wolff, H.P. (1956), Klin Wochenschr. 34, 309.

Yunice, A.A., Lindeman, R.D. (1975) Endocrinology: 97(5), 1263

APPENDIX.

Abbreviations used in the analysis of variance tables.

S.S.	.....	Sum of squares.
D.F.	.....	Degrees of freedom
M.S.	.....	Mean square
F.ratio.	.....	Variance ratio

FOOD INTAKE PER WEEK - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	2112833.3	3	704277.8	6.74	***
Sex	14896653.0	1	14896653.0	141.33	***
Diets	17876042.0	2	8938020.8	84.80	***
Weeks x sex	48086.7	3	16028.9	0.15	N.S.
Weeks x diets	135431.7	6	22571.9	0.21	N.S.
Sex x diets	330041.7	2	165020.8	1.57	N.S.
Weeks x sex x diets	223458.3	6	37243.1	0.35	N.S.
Error	10118403.3	96	105400.0		
Total	45740947.0	119			

FOOD INTAKE PER WEEK - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	853369.17	3	284456.39	4.9	**
Sex	37957501.00	1	37957501.00	655.9	*
Diets	152006.67	2	76003.333	1.3	N.S.
Weeks x sex	154242.50	3	51414.167	0.89	N.S.
Weeks x diets	21193.333	6	3532.2222	0.06	N.S.
Sex x diets	248806.700	2	1244403.3	21.5	***
Weeks x sex x diets	9300.000	6	1550.0000	0.027	N.S.
Error	5555682	96	57871.68		
Total	44952099	118			

ZINC INTAKE- PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	1337.24	3	445.746	4.55	**
Sex	11916.147	1	11916.147	3.00	N.S.
Diets	1723369.0	2	861684.52	28.00	*
Weeks x sex	64.3403	3	21.446776	0.21	N.S.
Weeks x diets	772.00	6	128.66678	1.31	N.S.
Sex x diets	6015.53	2	3007.767	30.70	***
Weeks x sex x diets	290.90067	6	148.483	1.51	N.S.
Error	9389.0	96	97.80		
Total	1752891.3	119			

ZINC INTAKE --PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	565.89066	3	188.63022	4.85	**
Sex	22957.80000	1	22957.8	590	N.S.
Diets	1458193.4	2	729096.72	1876	N.S.
Weeks x sex	96.067666	3	32.022555	0.82	N.S.
Weeks x diets	289.23131	6	48.205218	1.24	N.S.
Sex x diets	13519.305	2	6759.6523	173.9	***
Weeks x sex x diets	54.803339	6	9.1338898	0.24	N.S.
Error	3730.0	96	38.854		
Total	1499406.5	119			

GROWTH RATE PER WEEK - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	2547873.3	3	849291.11	2.26	N.S.
Sex	3563853.3	1	3563853.30	9.47	***
Diets	3152952.0	2	15766476.00	41.90	***
Weeks x sex	2248873.3	3	749624.44	1.99	N.S.
Weeks x diets	9376841.7	6	1562806.90	4.15	**
Sex x diets	2090151.7	2	1045075.80	2.78	N.S.
Weeks x sex x diets	3970041.7	6	661673.61	1.76	N.S.
Error	36121360.0	96	376264.17		
Total	63071947	119			

GROWTH RATE PER WEEK - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	85429.167	3	28476.389	0.37	N.S.
Sex	1380307.5	1	1380307.5	18.25	N.S.
Diets	11071215	2	5535607.5	73.2	N.S.
Weeks x sex	117229.17	3	39076.389	0.52	N.S.
Weeks x diets	529518.33	6	88253.056	1.17	N.S.
Sex x diets	1934865	2	967432.50	12.8	***
Weeks x sex x diets	93068.333	6	15511.389	0.205	N.S.
Error	7259962	96	75624.6		
Total	22471593	119			

FOOD CONVERSION EFFICIENCY - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	39.966897	3	13.320299	0.212	N.S.
Sex	475.61020	1	475.61020	7.57	**
Diets	7864.66	2	3932.3326	62.63	***
Weeks x sex	1018.1649	3	339.38831	5.41	N.S.
Weeks x diets	553.78083	6	92.296805	1.47	N.S.
Sex x diets	570.05335	2	285.02667	4.54	N.S.
Weeks x sex x diets	1473.6328	6	245.60547	3.91	**
Error	6027.46	96	62.786		
Total	18023.256	119			

FOOD CONVERSION EFFICIENCY - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	2345.1582	3	781.71940	2.86	N.S.
Sex	10280.455	1	10280.455	37	*
Diets	9831.747	2	4915.8735	18	N.S.
Weeks x sex	4678.9680	3	1559.6560	5.7	N.S.
Weeks x diets	4541.6304	6	756.9384	2.77	N.S.
Sex x diets	12666.467	2	6333.2336	23.2	N.S.
Weeks x sex x diets	9207.5541	6	1534.5924	5.6	***
Error	26152.1533	96	272.418		
Total	79704.133				

APPENDIX 4

PLASMA ZINC - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.0112692	3	0.0037564	2.17	N.S.
Sex	0.0414406	1	0.0414406	4.00	***
Diets	20.079564	2	10.039782	5816.8	***
Weeks x sex	0.0013758	3	0.0004586	0.0266	N.S.
Weeks x diets	0.0135184	6	0.0022531	1.3	N.S.
Sex x diets	0.0036516	2	0.0018258	1.05	N.S.
Weeks x sex x diets	0.0033217	6	0.0005536	0.32	N.S.
Error	0.16570	96	0.001726		
Total	20.319701	119			

PLASMA ZINC - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.0482291	3	0.0160764	2.59	N.S.
Sex	0.0323407	1	0.0323407	5.2	*
Diets	23.819782	2	11.909891	1915.8	***
Weeks x sex	0.0272492	3	0.0090831	1.46	N.S.
Weeks x diets	0.1592732	6	0.0265455	4.27	**
Sex x diets	0.0158066	2	0.0079033	1.27	N.S.
Weeks x sex x diets	0.0689534	6	0.0114922	1.849	N.S.
Error	0.59967	96	0.006216		
Total	24.768394	119			

ALKALINE PHOSPHOTASE ACTIVITY - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	16.092784	3	5.3642612	4.26	N.S.
Sex	6.806803	1	6.8068028	5.41	N.S.
Diets	771.94040	2	385.9702	306.9	***
Weeks x sex	6.5125175	3	2.1708392	1.73	N.S.
Weeks x diets	30.373173	6	5.0621955	4.03	**
Sex x diets	83.194194	2	41.597097	33.1	***
Weeks x sex x diets	13.386835	6	2.2311392	1.77	N.S.
Error	720.75	96	1.2578		
Total	1018.689	119			

ALKALINE PHOSPHATASE ACTIVITY - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	2.0155365	3	0.6718455	0.32	N.S.
Sex	3.5569625	1	3.5569625	1.679	N.S.
Diets	1100.6497	2	550.32484	259.8	***
Weeks x sex	2.4375364	3	0.8125121	0.38	N.S.
Weeks x diets	41.994174	6	6.999029	3.3	**
Sex x diets	16.983287	2	8.4916433	4.01	*
Weeks x sex x diets	16.637776	6	2.7729626	1.31	N.S.
Error	203.336	96	2.11808		
Total	1387.6114	119			

PLASMA PROTEIN CONCENTRATION - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	6.6747141	3	2.2249047	19.09	N.S.
Sex	1.5030411	1	1.5030411	12.90	N.S.
Diets	126.59973	2	63.299864	543.0	***
Weeks x sex	0.3835161	3	0.1278387	1.09	N.S.
Weeks x diets	13.694759	6	2.2824599	19.58	***
Sex x diets	1.945261	2	0.9726303	8.34	***
Weeks x sex x diets	0.3024121	6	0.0504020	0.43	N.S.
Error	11.191	96	0.1166		
Total	162.29087	119			

PLASMA PROTEIN CONCENTRATION - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.1862766	3	0.0620922	0.2	N.S.
Sex	1.9660802	1	1.9660802	6.28	N.S.
Diets	130.26893	2	65.134464	208.05	***
Weeks x sex	0.0133067	3	0.0044356	0.014	N.S.
Weeks x diets	30.268115	6	5.0446858	16.114	***
Sex x diets	12.920460	2	6.4602301	20.63	***
Weeks x sex x diets	0.1524331	6	0.0254055	0.081	N.S.
Error	30.0543	96	0.3130656		
Total	205.8386	119			

ALBUMIN - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.1368491	3	0.0456164	2.136	N.S.
Sex	0.0003008	1	0.0003008	0.014	N.S.
Diets	9.3308089	2	4.6654045	218.55	***
Weeks x sex	0.0036891	3	0.0012297	0.058	N.S.
Weeks x diets	0.4430485	6	0.0738414	3.46	*
Sex x diets	0.8541023	2	0.4270512	20	***
Weeks x sex x diets	0.0117983	6	0.0019664	0.092	N.S.
Error	2.049276	96	0.02134663		
Total	12.829276	119			

ALBUMIN - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.4572352	3	0.1524117	1.78	N.S.
Sex	0.6765014	1	0.6765014	7.92	N.S.
Diets	1.4031128	2	0.7015564	8.2	*
Weeks x sex	0.1870491	3	0.0623497	0.7	N.S.
Weeks x diets	3.3128817	6	0.5521470	6.47	***
Sex x diets	0.7249813	2	0.3624907	4.25	*
Weeks x sex x diets	0.6117584	6	0.1019597	1.195	N.S.
Error	8.194878	96	0.08536		
Total	15.568399	119			

TOTAL GLOBULIN - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	8.5509996	3	2.8503332	45.18	***
Sex	1.4963337	1	1.4963337	23.72	N.S.
Diets	200.60017	2	100.30008	1589.7	**
Weeks x sex	0.4389998	3	0.1463333	2.32	N.S.
Weeks x diets	18.734501	6	3.1224169	1.76	N.S.
Sex x diets	1.6321663	2	0.8160831	12.94	***
Weeks x sex x diets	0.4225	6	0.0704167	1.12	N.S.
Error	6.056	96	0.0630833		
Total	237.93167	119			

TOTAL GLOBULIN - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	1.7550001	3	0.5850000	0.265	N.S.
Sex	6.4403343	1	6.4403343	2.92	N.S.
Diets	147.15818	2	73.579090	33.37	*
Weeks x sex	0.66166662	3	0.2205554	0.102	N.S.
Weeks x diets	53.920499	6	8.9867499	4.08	**
Sex x diets	21.722167	2	10.861084	4.93	*
Weeks x sex x diets	1.5178339	6	0.2529723	0.115	N.S.
Error	43.52799	96	2.205		
Total	276.70368	119			

α_1-GLOBULIN - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.5100004	3	0.1700001	9.07	N.S.
Sex	1.4083335	1	1.4083335	75.13	N.S.
Diets	15.614002	2	7.8070008	416.6	***
Weeks x sex	0.0416667	3	0.0138889	0.74	N.S.
Weeks x diets	1.2040008	6	0.2006668	10.71	***
Sex x diets	2.2086668	2	1.1043334	58.9	***
Weeks x sex x diets	0.089335	6	0.0148889	0.795	N.S.
Error	1.799	96	0.0187396		
Total	22.872003	119			

α_1-GLOBULIN - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	1.0366668	3	0.3455556	0.9	N.S.
Sex	0.192000	1	0.192000	0.5	N.S.
Diets	8.9046660	2	4.4523330	11.75	***
Weeks x sex	0.9020000	3	0.3006667	0.78	N.S.
Weeks x diets	3.4213331	6	0.5702222	1.48	N.S.
Sex x diets	0.1500000	2	0.075000	0.2	N.S.
Weeks x sex x diets	2.212	6	0.3686667	0.06	
Error	36.92	96	0.3845		
Total	53.726665	119			

α2-GLOBULIN - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.014000	3	0.0046667	0.54	N.S.
Sex	0.208333	1	0.2083333	23.89	N.S.
Diets	1.3151668	2	0.6575834	80.10	N.S.
Weeks x sex	0.003000	3	0.001000	0.11	N.S.
Weeks x diets	0.1095000	6	0.018250	2.1	N.S.
Sex x diets	0.1971667	2	0.0985834	11.32	***
Weeks x sex x diets	0.0155000	6	0.0025833	0.297	N.S.
Error	0.83599	96	0.008708		
Total	2.698666	119			

α2-GLOBULIN - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	2.0463336	3	0.6821112	1.09	N.S.
Sex	0.4319999	1	0.4319999	0.69	N.S.
Diets	10.781167	2	5.3905834	8.66	***
Weeks x sex	1.6573333	3	0.5524444	0.881	N.S.
Weeks x diets	3.2041670	6	0.5340278	0.86	N.S.
Sex x diets	0.3705000	2	0.18525000	0.2	N.S.
Weeks x sex x diets	4.0721665	6	0.6786944	1.09	N.S.
Error	59.76	96	0.62247		
Total	81.883673	119			

β-GLOBULIN - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.90449167	3	0.3016389	18.56	N.S.
Sex	0.1020833	1	0.102833	6.28	N.S.
Diets	20.394498	2	10.197249	627.5	***
Weeks x sex	0.2069169	3	0.0689723	4.24	N.S.
Weeks x diets	1.6868335	6	0.2811389	17.3	*
Sex x diets	0.0101667	2	0.0050833	0.313	N.S.
Weeks x sex x diets	0.2618336	6	0.0436389	2.69	*
Error	1.56	96	0.01625		
Total	25.123249	119			

β-GLOBULIN - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.2655835	3	0.0885278	0.342	N.S.
Sex	0.3520836	1	0.3520836	1.36	N.S.
Diets	19.650501	2	9.8252504	37.9	*
Weeks x sex	0.6142497	3	0.2047499	0.79	N.S.
Weeks x diets	5.7341676	6	0.9556946	3.69	**
Sex x diets	0.3861666	2	0.1930833	0.75	N.S.
Weeks x sex x diets	1.1265009	6	0.1877502	0.72	N.S.
Error	24.86	96	0.25896		
Total	52.993255	119			

γ-GLOBULIN - PERIOD 1 (Weeks 3-6)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	1.5486667	3	0.5162222	22.94	N.S.
Sex	0.0120001	1	0.0120001	0.53	N.S.
Diets	21.192171	2	10.596085	470.79	***
Weeks x sex	0.1179999	3	0.0393333	1.75	N.S.
Weeks x diets	2.7898333	6	0.4649722	20.66	***
Sex x diets	0.1115000	2	0.0557500	2.48	N.S.
Weeks x sex x diets	0.2224999	6	0.0370833	1.65	N.S.
Error	2.16067	96	0.02250697		
Total	28.15867	119			

γ-GLOBULIN - PERIOD 2 (Weeks 9-12)

Source of variance	S.S.	D.F.	M.S.	F. ratio	Sig. level
Weeks	0.5495831	3	0.1831944	0.393	N.S.
Sex	0.7840833	1	0.7840833	302.1	***
Diets	28.857166	2	14.428583	30.96	*
Weeks x sex	1.14825	3	0.38275	0.82	N.S.
Weeks x diets	8.086167	6	1.3476945	2.89	*
Sex x diets	1.3711664	2	0.6855832	1.47	N.S.
Weeks x sex x diets	1.9775007	6	0.3295834	0.71	
Error	44.74	96	0.4660416		
Total	87.509915	119			

Treatment 1 - Animals fed on Zinc deficient diet - 0.9 mg.Zn./Kg.
2 - Animals fed on Zinc supplemented diet - 40 mg.Zn./Kg.
3 - Animals fed on Zinc deficient diet-0.9 mg.Zn./Kg. which were previously on Treatment 2
4 - Animals fed on Zinc supplemented diet, 40 mg.Zn./Kg., which were previously on Treatment 1
5 - Animals fed the same quantity of food as eaten by Group 1, but supplemented with 40 mg.Zn./Kg.
6 - Animals fed the same quantity of food as eaten by Group 3, but supplemented with 40 mg.Zn./Kg.

Weeks	Lamb No.	Treatment	Sex	Zinc in diet	Weekly mean			F.C.E.	Mean plasma values per week			Mean plasma values per week g/100 ml					
					Food intake	Zinc intake	Growth rate		Zinc	Protein	Alk.phospht.	Albumin	α1	α2	β-glob.	γ-glob.	Total globulin
				mg/kg	g	mg	g		µg/ml	g/100 ml	Sigma units						
3	255	1	2	0.9	6080	5.5	150	40.5	0.3	6.85	2.85	3.60	0.3	0.5	1.2	1.3	3.3
4	"	1	2	0.9	6300	5.7	300	21.0	0.26	7.70	2.47	3.38	0.4	0.6	1.7	1.6	4.3
5	"	1	2	0.9	6560	5.9	200	32.8	0.24	8.50	2.05	3.25	0.6	0.7	1.9	2.1	5.3
6	"	1	2	0.9	6380	5.8	300	21.3	0.27	9.48	1.67	3.10	0.7	0.7	2.3	2.7	6.4
9	"	4	2	40.0	6220	248.8	520	12.0	1.08	7.53	6.47	3.50	0.4	0.6	1.3	1.7	4.0
10	"	4	2	40.0	6300	252.0	680	9.3	1.14	6.68	7.52	3.65	0.2	0.6	0.9	1.3	3.0
11	"	4	2	40.0	6330	253.2	720	8.8	1.15	6.50	8.22	3.68	0.2	0.5	0.8	1.3	2.8
12	"	4	2	40.0	6350	254.0	700	9.1	1.17	6.28	8.50	3.68	0.2	0.5	0.7	1.2	2.6
3	256	1	2	0.9	6220	5.6	000	-	0.32	7.34	2.63	3.53	0.7	0.5	1.2	1.4	3.8
4	"	1	2	0.9	6630	6.0	250	26.5	0.28	7.83	2.35	3.45	0.8	0.6	1.3	1.7	4.4
5	"	1	2	0.9	6600	5.9	150	44.0	0.30	8.63	2.00	3.34	0.8	0.6	1.8	2.1	5.3
6	"	1	2	0.9	6710	6.0	250	27.0	0.26	9.50	1.50	3.18	0.9	0.6	2.2	2.6	6.3
9	"	4	2	40.0	6250	250.0	450	14.0	0.12	0.46	2.48	3.20	0.8	0.5	2.5	2.5	6.3
10	"	4	2	40.0	6300	252.0	700	9.0	1.09	8.43	3.37	3.28	0.8	0.5	1.9	2.0	5.2
11	"	4	2	40.0	6280	251.2	700	9.0	1.18	6.53	8.53	3.60	0.4	0.4	1.0	1.1	2.9
12	"	4	2	40.0	6350	254.0	650	10.0	1.20	6.20	8.57	3.65	0.4	0.4	0.8	1.0	2.6
3	257	1	2	0.9	6280	5.7	300	21.0	0.30	7.40	2.55	3.50	0.3	0.5	1.5	1.6	3.9
4	"	1	2	0.9	6320	5.7	280	22.6	0.35	7.68	2.37	3.36	0.6	0.7	1.5	1.5	4.3
5	"	1	2	0.9	6540	6.0	170	38.5	0.31	8.60	1.88	3.24	0.8	0.7	1.9	2.0	5.4
6	"	1	2	0.9	6660	6.0	300	22.2	0.25	9.35	1.44	3.13	1.1	0.7	2.1	2.3	6.2
9	"	4	2	40.0	6300	252.0	440	14.3	1.10	7.62	6.68	3.46	0.5	0.4	1.6	1.7	4.2
10	"	4	2	40.0	6300	252.0	620	10.2	1.16	6.68	7.48	3.60	0.3	0.5	0.9	1.4	3.1
11	"	4	2	40.0	6350	254.0	850	7.5	1.16	6.55	8.44	3.64	0.3	0.4	0.8	1.4	2.9
12	"	4	2	40.0	6400	256.0	550	11.6	1.18	6.35	8.33	3.68	0.2	0.4	0.8	1.3	2.7
3	258	1	2	0.9	5940	5.4	250	24.0	0.32	7.28	1.88	3.43	0.5	0.7	1.2	1.5	3.9
4	"	1	2	0.9	6180	5.6	200	31.0	0.36	7.75	1.65	3.30	0.7	0.8	1.3	1.7	4.5
5	"	1	2	0.9	6320	5.7	150	42.1	0.32	8.52	1.43	3.25	0.8	0.7	1.8	2.0	5.3
6	"	1	2	0.9	6430	5.8	250	26.0	0.30	9.30	1.26	3.25	1.0	0.7	2.0	2.4	6.1
9	"	4	2	40.0	6200	248.0	600	10.3	1.13	7.30	6.72	3.60	0.5	0.5	1.1	1.6	3.7
10	"	4	2	40.0	6280	251.0	600	10.5	1.18	6.65	7.78	3.65	0.3	0.3	1.0	1.4	3.0
11	"	4	2	40.0	6380	255.0	750	8.5	1.15	6.49	8.35	3.75	0.2	0.4	0.8	1.3	2.7
12	"	4	2	40.0	6350	254.0	650	9.8	1.16	6.25	8.24	3.73	0.2	0.5	0.8	1.0	2.5
3	259	1	2	0.9	5900	5.3	200	29.5	0.28	7.30	2.35	3.45	0.5	0.5	1.3	1.6	3.9
4	"	1	2	0.9	6230	5.6	250	25.0	0.29	7.80	2.00	3.30	0.7	0.6	1.4	1.8	4.5
5	"	1	2	0.9	6410	5.8	150	43.0	0.26	8.68	1.67	3.20	0.7	0.6	1.9	2.3	5.5
6	"	1	2	0.9	6470	5.8	250	26.0	0.28	9.45	1.30	3.10	0.7	0.6	2.3	2.8	6.4
9	"	4	2	40.0	6200	248.0	500	12.4	1.13	7.24	6.55	3.48	0.6	0.6	1.6	2.0	3.8
10	"	4	2	40.0	6200	248.0	600	10.3	1.15	6.80	7.53	3.58	0.4	0.5	0.9	1.4	3.2
11	"	4	2	40.0	6350	254.0	850	7.5	1.17	6.50	8.66	3.65	0.2	0.4	0.9	1.4	2.9
12	"	4	2	40.0	6360	254.0	600	10.6	1.19	6.28	8.28	3.68	0.3	0.4	0.9	1.0	2.6

Weeks	Lamb No.	Treat-ment	Sex	Zinc in diet	Weekly mean			F.C.E.	Mean plasma values per week			Mean plasma values per week g/100 ml					
					Food intake	Zinc intake	Growth rate		Zinc	Protein	Alk.phospht.	Albumin	∝1	∝2	β-glob.	γ-glob.	Total globulin
				mg/kg	g	mg	g		g/ml	g/100 ml	Sigma units						
3	250	2	2	40.0	7250	290.0	1200	6.1	1.20	6.14	10.00	3.75	0.2	0.5	0.7	1.0	2.4
4	"	2	2	40.0	7200	288.0	1400	5.2	1.22	6.10	9.80	3.70	0.2	0.5	0.7	1.0	2.4
5	"	2	2	40.0	7380	295.0	1150	6.4	1.16	6.20	9.55	3.78	0.2	0.4	0.8	1.0	2.4
6	"	2	2	40.0	7240	290.0	750	9.7	1.16	6.23	9.40	3.80	0.2	0.4	0.8	1.0	2.4
9	"	3	2	0.9	6580	5.9	300	22.0	0.28	6.30	2.35	3.80	0.2	0.5	0.8	1.0	2.5
10	"	3	2	0.9	6630	6.0	50	132.6	0.16	6.55	1.05	3.73	0.3	0.5	0.9	1.1	2.8
11	"	3	2	0.9	6700	6.0	250	26.8	0.18	7.50	0.90	3.64	0.3	0.5	1.4	1.7	3.9
12	"	3	2	0.9	6700	6.0	300	22.3	0.23	8.24	0.95	3.60	0.3	0.5	1.6	2.2	4.6
3	251	2	2	40.0	7200	288.0	1150	6.3	1.15	5.90	8.48	3.57	0.2	0.5	0.7	0.9	2.3
4	"	2	2	40.0	7280	291.0	1700	4.3	1.18	6.00	9.20	3.60	0.2	0.5	0.7	1.0	2.4
5	"	2	2	40.0	7400	296.0	930	8.0	1.10	6.18	9.43	3.83	0.2	0.5	0.7	1.0	2.3
6	"	2	2	40.0	7250	290.0	900	8.1	1.12	6.20	9.37	3.78	0.2	0.4	0.8	1.0	2.4
9	"	3	2	0.9	6600	5.9	200	33.0	0.36	6.30	6.73	3.84	0.2	0.5	0.8	1.0	2.5
10	"	3	2	0.9	6650	6.0	50	133.0	0.38	6.45	4.68	3.87	0.3	0.5	0.8	1.0	2.6
11	"	3	2	0.9	6700	6.0	330	20.3	0.27	7.40	1.15	3.28	0.3	0.5	1.5	1.8	4.1
12	"	3	2	0.9	6740	6.1	170	39.7	0.25	7.85	1.20	3.25	0.3	0.5	1.7	2.1	4.6
3	252	2	2	40.0	7180	287.0	980	7.3	1.22	6.38	8.40	3.94	0.2	0.5	0.8	0.9	2.4
4	"	2	2	40.0	7280	291.0	1750	4.2	1.18	6.25	8.36	3.86	0.2	0.5	0.7	1.0	2.4
5	"	2	2	40.0	7350	294.0	870	8.5	1.16	6.30	8.29	3.79	0.2	0.5	0.8	1.0	2.5
6	"	2	2	40.0	7300	292.0	900	8.1	1.15	6.28	8.26	3.79	0.2	0.5	0.8	1.0	2.5
9	"	3	2	0.9	6500	6.0	250	26.0	0.25	7.60	2.00	3.72	0.5	0.4	1.5	1.5	3.9
10	"	3	2	0.9	6600	5.9	180	36.7	0.18	8.84	1.35	3.64	0.8	0.6	1.8	2.0	5.2
11	"	3	2	0.9	6690	6.0	270	24.8	0.20	9.38	1.40	3.37	1.2	0.7	1.8	2.3	6.0
12	"	3	2	0.9	6780	6.1	50	135.6	0.24	9.70	1.10	3.18	1.2	0.7	2.1	2.5	6.5
3	253	2	2	40.0	7300	292.0	750	9.7	1.15	6.15	8.82	3.67	0.2	0.5	0.8	1.0	2.5
4	"	2	2	40.0	7300	292.0	1600	4.6	1.18	6.00	8.70	3.67	0.2	0.4	0.7	1.0	2.3
5	"	2	2	40.0	7380	295.0	1000	7.4	1.15	6.30	8.58	3.86	0.2	0.4	0.8	1.0	2.4
6	"	2	2	40.0	7250	290.0	900	8.1	1.20	6.35	8.64	3.94	0.2	0.4	0.8	1.0	2.4
9	"	3	2	0.9	6650	6.0	400	16.6	0.30	7.40	1.75	3.73	0.4	0.6	1.2	1.5	3.7
10	"	3	2	0.9	6680	6.0	100	66.8	0.25	8.50	1.22	3.54	0.4	0.6	1.8	2.2	5.0
11	"	3	2	0.9	6740	6.1	210	32.1	0.28	9.00	1.35	3.28	0.4	0.7	2.1	2.5	5.7
12	"	3	2	0.9	6800	6.1	290	23.5	0.26	9.23	1.23	3.21	0.5	0.7	2.2	2.7	6.0
3	254	2	2	40.0	7250	290.0	1200	6.1	1.22	6.45	9.68	3.91	0.3	0.4	0.8	1.0	2.5
4	"	2	2	40.0	7300	292.0	1500	4.9	1.18	6.50	9.35	3.95	0.2	0.4	0.9	1.0	2.5
5	"	2	2	40.0	7420	297.0	1050	7.1	1.16	6.38	9.32	3.80	0.2	0.4	1.0	1.0	2.6
6	"	2	2	40.0	7250	290.0	780	9.3	1.18	6.30	9.58	3.76	0.2	0.4	0.9	1.0	2.5
9	"	3	2	0.9	6680	6.0	450	14.9	0.26	7.45	1.80	3.73	0.5	0.8	1.0	1.4	3.7
10	"	3	2	0.9	6700	6.0	50	134.0	0.22	8.63	1.30	3.64	0.7	0.9	1.6	1.8	5.0
11	"	3	2	0.9	6750	6.1	280	24.1	0.28	9.25	1.50	3.45	0.9	1.0	1.7	2.2	5.8
12	"	3	2	0.9	6800	6.1	120	56.7	0.28	9.25	1.48	3.36	1.2	0.9	1.6	2.2	5.9

Weeks	Lamb No.	Treatment	Sex	Zinc in diet	Weekly mean			F.C.E.	Mean plasma values per week			Mean plasma values per week g/100 ml					
					Food intake	Zinc intake	Growth rate		Zinc	Protein	Alk.phospht.	Albumin	∝1	∝2	β-glob.	γ-glob.	Total globulin
				mg/kg	g	mg	g		g/ml	g/100 ml	Sigma units						
3	260	5	2	40.0	6080	243.0	950	6.4	1.15	6.20	8.38	3.81	0.2	0.4	0.8	1.0	2.4
4	"	5	2	40.0	6300	252.0	950	6.6	1.13	6.25	8.70	3.86	0.2	0.4	0.8	1.0	2.4
5	"	5	2	40.0	6560	262,0	900	7.3	1.16	6.15	8.85	3.68	0.2	0.4	0.9	1.0	2.5
6	"	5	2	40.0	6380	255.0	1200	5.3	1.15	6.23	8.65	3.74	0.2	0.4	0.9	1.0	2.5
9	"	6	2	40.0	6580	263.0	550	12.0	1.16	6.35	8.55	3.91	0.2	0.4	0.8	1.0	2.4
10	"	6	2	40.0	6630	265.0	650	10.2	1.18	6.28	8.67	3.77	0.2	0.4	0.9	1.0	2.5
11	"	6	22	40.0	6700	268.0	700	9.6	1.16	6.30	8.50	3.90	0.2	0.5	0.7	1.0	2.4
12	"	6	2	40.0	6700	268.0	600	11.2	1.15	6.30	8.50	3.86	0.2	0.4	0.8	1.0	2.4
3	261	5	2	40.0	6220	249.0	860	7.2	1.18	6.28	8.47	3.82	0.2	0.4	0.9	1.0	2.5
4	"	5	2	40.0	6630	265.0	900	7.4	1.17	6.32	8.38	3.79	0.2	0.5	0.8	1.0	2.5
5	"	5	2	40.0	6600	264.0	1000	6.6	1.19	6.30	8.64	3.87	0.2	0.5	0.7	1.0	2.4
6	"	5	2	40.0	6710	268.0	1100	6.1	1.18	6.28	8.57	3.78	0.2	0.5	0.8	1.0	2.5
9	"	6	2	40.0	6600	264.0	600	11.0	1.15	6.28	8.33	3.81	0.2	0.4	0.9	1.0	2.5
10	"	6	2	40.0	6650	266.0	500	13.3	1.16	6.34	8.50	3.80	0.2	0.5	0.8	1.0	2.5
11	"	6	2	40.0	6700	268.0	850	8.0	1.18	6.24	8.53	3.81	0.2	0.4	0.8	1.0	2.4
12	"	6	2	40.0	6740	270.0	700	9.6	1.14	6.25	8.48	3.81	0.2	0.4	0.8	1.0	2.4
3	262	55	2	40.0	6280	251.0	850	7.4	1.16	6.20	8.67	3.63	0.2	0.5	0.9	1.0	2.6
4	"	5	2	40.0	6320	253.0	950	6.7	1.15	6.23	8.84	3.65	0.2	0.5	0.9	1.0	2.6
5	"	5	2	40.0	6540	262.0	1050	6.2	1.13	6.15	8.68	3.60	0.2	0.5	0.9	1.0	2.5
6	"	5	2	40.0	6660	266.0	1100	6.1	1.17	6.15	8.55	3.65	0.2	0.5	0.8	1.0	2.5
9	"	6	2	40.0	6500	260.0	450	14.5	1.16	6.20	8.40	3.68	0.2	0.5	0.8	1.0	2.5
10	"	6	2	40.0	6600	264.0	650	10.2	1.19	6.25	8.42	3.70	0.2	0.5	0.9	1.0	2.5
11	"	6	2	40.0	6690	268.0	770	8.7	1.14	6.18	8.56	3.70	0.2	0.5	0.8	1.0	2.5
12	"	6	2	40.0	6780	271.0	780	8.7	1.13	6.20	8.55	3.68	0.2	0.5	0.8	1.0	2.5
3	263	5	2	40.0	5940	238.0	950	6.3	1.17	6.42	10.15	3.89	0.2	0.4	0.9	1.0	2.5
4	"	5	2	40.0	6180	247.0	900	6.9	1.15	6.40	9.88	3.94	0.2	0.4	0.9	1.0	2.5
5	"	5	2	40.0	6320	253.0	1050	6.0	1.18	6.38	9.75	3.95	0.2	0.4	0.8	1.0	2.4
6	"	5	2	40.0	6430	257.0	1050	6.1	1.18	6.35	9.60	3.81	0.2	0.4	0.9	1.0	2.5
9	"	6	2	40.0	6650	266.0	1100	6.1	1.16	6.35	9.50	3.81	0.2	0.4	0.9	1.0	2.5
10	"	6	2	40.0	6680	267.0	700	9.6	1.13	6.25	9.08	3.75	0.2	0.4	0.9	1.0	2.5
11	"	6	2	40.0	6740	270.0	700	9.6	1.12	6.28	9.33	3.77	0.2	0.4	0.9	1.0	2.5
12	"	6	2	40.0	6800	272.0	550	12.4	1.15	6.25	9.25	3.75	0.2	0.4	0.9	1.0	2.5
3	264	5	2	40.0	5900	236.0	950	6.2	1.16	6.10	10.30	3.72	0.2	0.5	0.7	1.0	2.4
4	"	5	2	40.0	6230	249.0	900	6.9	1.20	6.00	9.90	3.66	0.2	0.5	0.6	1.0	2.3
5	"	5	2	40.0	6410	256.0	1100	5.8	1.18	6.22	9.85	3.79	0.2	0.5	0.7	1.0	2.4
6	"	5	2	40.0	6470	259.0	1100	5.9	1.15	6.23	10.40	3.80	0.2	0.5	0.7	1.0	2.4
9	"	6	2	40.0	6680	267.0	1200	5.6	1.15	6.28	10.05	3.83	0.2	0.5	0.8	1.0	2.4
10	"	6	2	40.0	6700	268.0	650	10.3	1.14	6.20	9.45	3.78	0.2	0.5	0.7	1.0	2.4
11	"	6	2	40.0	6750	270.0	710	9.5	1.17	6.23	9.67	3.80	0.2	0.5	0.7	1.0	2.4
12	"	6	22	40.0	6800	272.0	640	10.6	1.16	6.18	9.72	3.77	0.2	0.5	0.7	1.0	2.4

Weeks	Lamb No.	Treatment	Sex	Zinc in diet	Weekly mean			F.C.E.	Mean plasma values per week			Mean plasma values per week g/100 ml					
					Food intake	Zinc intake	Growth rate		Zinc	Protein	Alk.phospht.	Albumin	α1	α2	β-glob.	γ-glob.	Total globulin
				mg/kg	g	mg	g		g/ml	g/100 ml	Sigma units						
3	724	1	1	0.9	3900	3.5	400	9.8	0.29	7.11	7.30	3.20	1.2	0.6	0.9	1.2	3.9
4	"	1	1	0.9	5240	4.7	200	26.2	0.27	8.08	5.20	3.18	1.3	0.9	1.3	1.4	4.9
5	"	1	1	0.9	5750	5.2	400	14.4	0.27	8.55	3.00	3.15	1.4	0.7	1.4	1.9	5.4
6	"	1	1	0.9	5840	5.3	100	58.4	0.23	9.23	2.30	3.10	1.6	0.7	1.6	2.2	6.1
9	"	4	1	40.0	5130	205.0	800	6.4	0.97	6.59	7.40	3.29	0.8	0.7	0.6	1.2	3.4
10	"	4	1	40.0	5370	215.0	900	6.0	0.98	6.30	7.40	3.40	0.6	0.8	0.6	0.9	2.9
11	"	4	1	40.0	5720	229.0	1200	4.8	1.12	5.70	8.40	3.47	0.4	0.6	0.5	0.7	2.2
12	"	4	1	40.0	5480	219.0	1700	3.2	1.15	5.45	8.20	3.57	0.3	0.5	0.4	0.7	1.9
3	796	1	1	0.9	6200	5.6	300	20.7	0.32	8.27	7.00	3.35	1.2	0.6	1.4	1.7	4.9
4	"	1	1	0.9	5680	5.1	200	28.4	0.33	8.77	6.00	3.23	1.4	0.8	1.6	1.7	5.5
5	"	1	1	0.9	5840	5.3	400	14.6	0.32	9.13	4.20	3.18	1.6	0.9	1.6	1.8	5.9
6	"	1	1	0.9	6000	5.4	200	30.0	0.24	9.71	3.00	3.16	1.8	0.9	1.7	2.1	6.5
9	"	4	1	40.0	6000	240.0	1200	5.0	0.97	8.02	7.30	3.34	0.9	0.7	1.4	1.6	4.7
10	"	4	1	40.0	5800	232.0	1900	3.1	1.05	7.78	7.60	3.54	0.7	0.8	1.3	1.4	4.2
11	"	4	1	40.0	5700	228.0	1200	4.8	1.09	6.83	8.50	3.68	0.5	0.6	1.1	0.9	3.1
12	"	4	1	40.0	5800	232.0	1000	5.8	1.17	6.53	8.40	3.88	0.3	0.6	0.9	0.8	2.6
3	781	1	1	0.9	6200	5.6	400	15.5	0.30	7.89	7.50	3.30	0.8	0.8	1.4	1.6	4.6
4	"	1	1	0.9	5800	5.2	300	19.3	0.25	8.85	6.30	3.26	1.4	0.9	1.5	1.8	5.6
5	"	1	1	0.9	6000	5.4	-200	-30.0	0.23	9.63	3.80	3.15	1.8	1.0	1.7	2.0	6.5
6	"	1	1	0.9	6000	5.4	500	12.0	0.22	10.23	2.70	3.00	2.0	1.2	1.8	2.3	7.2
9	"	4	1	40.0	5600	224.0	1300	4.3	0.98	7.70	7.30	3.42	0.9	0.8	1.3	1.2	4.3
10	"	4	1	40.0	5700	228.0	1200	4.8	1.06	6.93	7.90	3.65	0.7	0.7	0.9	0.9	3.3
11	"	4	1	40.0	6100	244.0	1000	6.1	1.14	6.59	8.90	3.71	0.4	0.8	0.8	0.8	2.9
12	"	4	1	40.0	5800	232.0	1600	3.6	1.14	6.26	8.10	3.78	0.4	0.7	0.7	0.7	2.5
3	839	1	1	0.9	5700	5.1	400	14.3	0.28	8.31	7.00	3.67	0.6	0.8	1.6	1.6	4.6
4	"	1	1	0.9	5900	5.3	600	9.8	0.28	8.52	5.80	3.32	0.8	0.6	1.8	2.0	5.2
5	"	1	1	0.9	5600	5.0	400	14.0	0.24	9.40	3.60	3.20	1.3	0.6	2.0	2.2	6.2
6	"	1	1	0.9	5800	5.2	200	29.0	0.21	9.46	2.40	3.16	1.5	0.7	1.9	2.2	6.3
9	"	4	1	40.0	5100	204.0	800	6.4	1.08	7.42	7.50	3.37	0.8	0.7	1.0	1.5	4.0
10	"	4	1	40.0	5400	216.0	1000	5.4	1.08	6.65	7.80	3.59	0.6	0.8	0.8	0.8	3.1
11	"	4	1	40.0	5200	208.0	1400	3.7	1.15	6.50	8.60	3.80	0.4	0.7	0.8	0.8	2.7
12	"	4	1	40.0	5800	232.0	1300	4.5	1.18	6.40	8.00	3.92	0.4	0.6	0.5	1.0	2.5
3	823	1	1	0.9	5800	5.2	400	14.5	0.30	7.68	6.90	3.18	0.8	0.6	1.5	1.6	4.5
4	"	1	1	0.9	5600	5.0	100	56.0	0.26	7.88	5.40	3.00	0.9	0.6	1.7	1.7	4.9
5	"	1	1	0.9	5800	5.2	500	11.6	0.25	8.33	3.70	2.90	1.1	0.6	1.9	1.8	5.4
6	"	1	1	0.9	5900	5.3	0	0	0.22	8.59	2.80	2.84	1.1	0.6	1.9	2.1	5.7
9	"	4	1	40.0	5400	216.0	1100	4.9	1.05	7.50	7.70	3.20	0.7	0.6	1.4	1.6	4.3
10	"	4	1	40.0	5700	228.0	800	7.1	1.10	6.40	7.50	3.45	0.5	0.8	0.7	0.9	2.9
11	"	4	1	40.0	5300	212.0	1400	3.8	1.14	5.80	8.50	3.58	0.3	0.6	0.6	0.7	2.2
12	"	4	1	40.0	5600	224.0	1300	4.3	1.16	5.75	8.10	3.65	0.4	0.6	0.4	0.7	2.1

RECORDED DATA (Continued)

Appendix 18

Weeks	Lamb No.	Treatment	Sex	Zinc in diet	Weekly mean			F.C.E.	Mean plasma values per week			Mean plasma values per week g/100 ml					
					Food intake	Zinc intake	Growth rate		Zinc	Protein	Alk.phospht.	Albumin	α1	α2	β-glob.	γ-glob.	Total globulin
				mg/kg	g	mg	g		g/ml	g/100 ml	Sigma units						
3	750	2	1	40.0	6900	276.0	1200	5.8	1.13	6.18	9.30	3.70	0.3	0.6	0.7	0.9	2.5
4	"	2	1	40.0	6400	256.0	1700	3.8	1.16	6.22	9.50	3.82	0.2	0.5	0.8	0.9	2.4
5	"	2	1	40.0	6800	272.0	2600	2.6	1.18	6.20	9.40	3.82	0.3	0.6	0.7	0.9	2.4
6	"	2	1	40.0	6600	264.0	1200	5.5	1.21	6.19	9.80	3.81	0.2	0.6	0.7	0.9	2.4
9	"	3	1	0.9	5200	4.7	600	8.7	0.15	8.25	1.80	3.18	0.6	0.9	1.8	1.8	5.1
10	"	3	1	0.9	5400	4.9	-300	-18.0	0.16	8.92	1.00	2.90	0.8	1.1	1.7	2.4	6.0
11	"	3	1	0.9	5500	5.0	300	18.3	0.18	9.66	0.90	2.80	0.9	1.3	2.0	2.7	6.8
12	"	3	1	0.9	5700	5.1	200	28.5	0.18	9.75	1.10	2.73	0.9	1.5	2.3	2.7	7.3
3	860	2	1	40.0	5800	232.0	1200	4.8	1.22	7.53	9.00	4.38	0.3	0.8	0.8	1.2	3.1
4	"	2	1	40.0	6300	252.0	2500	2.5	1.10	7.32	8.80	4.42	0.3	0.7	0.7	1.2	2.9
5	"	2	1	40.0	6200	248.0	1700	3.7	1.05	7.41	9.20	4.45	0.3	0.7	0.7	1.2	2.9
6	"	2	1	40.0	6400	256.0	1600	4.0	1.10	6.81	10.00	4.21	0.2	0.5	0.7	1.2	2.6
9	"	3	1	0.9	5000	4.5	300	16.7	0.18	8.62	1.60	3.75	0.6	0.8	1.2	2.3	4.9
10	"	3	1	0.9	5200	4.7	-700	-7.4	0.16	9.48	1.30	3.38	0.9	1.2	1.7	2.3	6.1
11	"	3	1	0.9	5600	5.0	300	18.7	0.20	9.75	1.10	3.10	1.0	1.3	1.9	2.5	6.6
12	"	3	1	0.9	5700	5.1	-200	-28.5	0.18	9.90	0.80	2.95	1.2	1.4	2.0	2.4	6.9
3	851	2	1	40.0	6000	240.0	1000	6.0	1.14	6.40	6.80	3.90	0.3	0.6	0.8	0.8	2.5
4	"	2	1	40.0	6500	260.0	3300	2.0	1.10	6.33	7.20	3.80	0.2	0.6	0.7	1.0	2.5
5	"	2	1	40.0	6800	272.0	2400	3.0	1.05	6.29	6.50	3.87	0.3	0.5	0.7	0.9	2.4
6	"	2	1	40.0	6700	268.0	1000	6.7	1.08	6.19	7.40	3.82	0.2	0.6	0.7	0.9	2.4
9	"	3	1	0.9	4800	4.3	200	24.0	0.18	8.57	1.40	3.65	0.5	0.8	1.7	1.9	4.9
10	"	3	1	0.9	5500	5.0	1600	3.4	0.15	9.27	1.50	3.57	0.7	1.2	1.8	2.0	5.7
11	"	3	1	0.9	5200	4.7	400	13.0	0.16	9.84	1.20	3.34	0.8	1.4	2.0	2.3	6.5
12	"	3	1	0.9	5400	4.9	0	0	0.20	10.18	0.90	3.00	1.0	1.4	2.2	2.6	7.2
3	859	2	1	40.0	5800	232.0	900	6.5	1.17	6.40	8.00	3.92	0.2	0.6	0.7	1.0	2.5
4	"	2	1	40.0	6500	260.0	2100	3.1	1.12	6.47	7.60	3.93	0.2	0.7	0.7	0.9	2.5
5	"	2	1	40.0	6500	260.0	2300	3.0	1.13	6.43	7.80	3.95	0.2	0.6	0.7	1.0	2.5
6	"	2	1	40.0	6800	272.0	1800	3.8	1.18	6.81	8.40	4.21	0.3	0.7	0.8	0.8	2.6
9	"	3	1	0.9	5300	4.8	300	17.7	0.22	8.62	1.40	4.00	0.6	1.1	1.3	1.6	4.6
10	"	3	1	0.9	5100	4.6	-200	-25.5	0.18	9.35	1.00	3.85	0.9	1.3	1.6	1.7	5.5
11	"	3	1	0.9	5000	4.5	-300	-16.7	0.15	9.95	0.90	3.54	1.1	1.4	1.9	2.0	6.4
12	"	3	1	0.9	5300	4.8	200	26.5	0.18	10.40	1.00	3.20	1.2	1.4	2.1	2.5	7.2
3	708	2	1	40.0	6400	256.0	1600	4.0	1.12	6.67	8.60	4.12	0.3	0.6	0.7	0.9	2.5
4	"	2	1	40.0	6400	256.0	2200	2.9	1.18	6.71	8.30	4.14	0.2	0.5	0.7	1.2	2.6
5	"	2	1	40.0	6500	260.0	3000	2.2	1.20	6.73	8.50	4.16	0.2	0.6	0.7	1.1	2.6
6	"	2	1	40.0	6400	256.0	1200	5.3	1.16	6.74	8.50	4.12	0.2	0.5	0.8	1.1	2.6
9	"	3	1	0.9	5500	5.0	-100	-55.0	0.18	7.45	1.50	3.65	0.6	0.9	1.1	1.2	3.8
10	"	3	1	0.9	5800	5.2	500	11.6	0.16	8.05	1.20	3.40	0.9	1.0	1.3	1.5	4.6
11	"	3	1	0.9	5400	4.9	200	27.0	0.18	9.10	1.10	3.25	1.2	1.3	1.4	1.9	5.8
12	"	3	1	0.9	5500	5.0	700	7.9	0.21	9.35	1.20	3.00	1.4	1.6	1.4	2.0	6.3

RECORDED DATA (Continued)

Weeks	Lamb No,	Treat-ment	Sex	Zinc in diet	Weekly mean			F.C.E.	Mean plasma values per week			Mean plasma values per week g/100 ml					
					Food intake	Zinc intake	Growth rate		Zinc	Protein	Alk.phospht.	Albumin	α1	α2	β-glob.	γ-glob.	Total globulin
				mg/kg	g	mg	g		g/ml	g/100 ml	Sigma units						
3	718	5	1	40.0	3900	156	600	6.5	1.18	6.14	8.8	3.65	0.2	0.5	0.8	1.0	2.5
4	"	5	1	40.0	5240	210	900	5.8	1.18	6.20	8.6	3.70	0.2	0.5	0.8	1.0	2.5
5	"	5	1	40.0	5750	230	1700	3.4	1.19	6.17	8.3	3.73	0.2	0.5	0.8	0.9	2.4
6	"	5	1	40.0	5840	234	1300	4.5	1.18	6.12	8.1	3.68	0.2	0.4	0.8	1.0	2.4
9	"	6	1	40.0	5200	208	850	6.1	1.20	6.13	9.0	3.66	0.2	0.4	0.9	1.0	2.5
10	"	6	1	40.0	5400	216	900	6.0	1.18	6.22	8.6	3.73	0.3	0.4	0.8	1.0	2.5
11	"	6	1	40.0	5500	220	650	8.5	1.16	6.34	8.8	3.72	0.3	0.4	0.9	1.0	2.6
12	"	6	1	40.0	5700	228	850	6.7	1.18	6.27	8.5	3.70	0.2	0.4	1.0	1.0	2.6
3	722	5	1	40.0	6200	248	300	20.7	1.12	6.42	9.4	3.70	0.2	0.5	0.8	1.2	2.7
4	"	5	1	40.0	5680	227	800	7.1	1.08	6.42	10.2	3.65	0.2	0.5	0.8	1.3	2.8
5	"	5	1	40.0	5840	234	1000	5.8	1.10	6.37	10.0	3.70	0.2	0.4	0.7	1.4	2.7
6	"	5	1	40.0	6000	240	1600	3.8	1.10	6.39	9.5	3.70	0.2	0.4	0.7	1.4	2.7
9	"	6	1	40.0	5000	200	750	6.7	1.08	6.44	9.0	3.60	0.2	0.4	0.8	1.4	2.8
10	"	6	1	40.0	5200	208	1250	4.2	1.08	6.37	9.4	3.70	0.2	0.5	0.7	1.3	2.7
11	"	6	1	40.0	5600	224	400	14.0	1.10	6.47	9.2	3.70	0.2	0.3	0.7	1.6	2.8
12	"	6	1	40.0	5700	228	650	8.8	1.08	6.42	9.5	3.70	0.2	0.5	0.8	1.2	2.7
3	734	5	1	40.0	6200	248	400	15.5	1.18	5.37	5.6	3.50	0.2	0.4	0.6	0.7	1.9
4	"	5	1	40.0	5800	232	700	8.3	1.17	5.42	5.8	3.50	0.3	0.4	0.6	0.7	2.0
5	"	5	1	40.0	6000	240	1200	5.0	1.18	5.41	4.8	3.55	0.2	0.4	0.7	0.7	2.0
6	"	5	1	40.0	6000	240	3200	2.0	1.17	5.41	5.0	3.58	0.2	0.4	0.7	0.7	2.0
9	"	6	1	40.0	4800	192	980	4.9	1.18	5.23	5.6	3.45	0.2	0.3	0.7	0.8	2.0
10	"	6	1	40.0	5500	220	800	6.9	1.15	5.34	5.8	3.55	0.2	0.4	0.6	0.8	2.0
11	"	6	1	40.0	5200	208	600	8.7	1.14	5.50	5.7	3.60	0.2	0.4	0.6	0.8	2.0
12	"	6	1	40.0	5400	216	650	8.3	1.16	5.44	5.6	3.55	0.2	0.3	0.7	0.8	2.0
3	788	5	1	40.0	5700	228	800	7.1	1.06	6.45	9.4	3.65	0.2	0.4	1.0	1.2	2.8
4	"	5	1	40.0	5900	236	500	11.8	1.11	6.35	8.5	3.60	0.2	0.4	1.0	1.2	2.8
5	"	5	1	40.0	5600	224	1700	3.3	1.08	6.32	8.9	3.65	0.2	0.4	0.9	1.2	2.7
6	"	5	1	40.0	5800	232	3100	2.0	1.08	6.25	8.6	3.60	0.2	0.3	1.0	1.2	2.7
9	"	6	1	40.0	5300	212	770	6.9	1.02	6.28	8.8	3.90	0.3	0.4	0.7	1.0	2.4
10	"	6	1	40.0	5100	204	550	9.3	1.06	6.20	8.6	3.85	0.3	0.5	0.6	1.0	2.4
11	"	6	1	40.0	5000	200	1200	4.2	1.08	6.15	8.4	3.80	0.2	0.5	0.8	0.9	2.4
12	"	6	1	40.0	5300	212	700	7.6	1.06	6.25	8.5	3.80	0.2	0.4	0.9	1.0	2.5
3	809	5	1	40.0	5800	232	300	19.3	1.03	6.32	9.5	3.90	0.2	0.4	0.8	1.0	2.4
4	"	5	1	40.0	5600	224	900	6.2	1.00	6.17	10.0	3.80	0.2	0.5	0.7	1.0	2.4
5	"	5	1	40.0	5800	232	1600	3.6	1.05	6.21	9.3	3.90	0.3	0.4	0.7	0.9	2.3
6	"	5	1	40.0	5900	236	2400	2.5	1.00	6.24	9.5	3.90	0.2	0.4	0.7	1.0	2.3
9	"	6	1	40.0	5500	220	1100	5.0	1.04	6.18	10.0	3.80	0.2	0.4	0.8	1.0	2.4
10	"	6	1	40.0	5800	232	500	11.6	1.08	6.25	10.3	3.90	0.2	0.4	0.8	1.0	2.4
11	"	6	1	40.0	5400	216	800	6.8	1.06	6.35	10.2	3.90	0.3	0.5	0.7	1.0	2.5
12	"	6	1	40.0	5500	220	900	6.1	1.06	6.30	9.8	3.90	0.3	0.5	0.6	1.0	2.4

First two weeks of each period in trial of zinc deficiency versus zinc normal

♂ = 1 ♀ = 2

Weeks	Lamb No.	Treat-ment	Sex	Weekly mean				F.C.E.	Plasma values per week			Plasma values per week g/100 ml				
				Zinc in diet	Food intake	Zinc intake	Growth rate		Zinc	Protein	Alk.phosph.	Albumin	α1 α2	β-glob.	γ-glob.	Total globulin
				mg/kg	g	mg	g		µg/ml	g/100 ml	Sigma units					
1	255	1	2	0.9	6300	5.7	000	63.0	0.38	6.2	8.05	3.72	0.2 0.5	0.8	1.0	2.5
2	"	1	2	0.9	6100	5.5	250	24.4	0.27	6.5	5.43	3.80	0.2 0.4	1.1	1.0	2.7
7	"	4	2	40.0	6400	256.0	500	12.8	0.94	9.39	2.36	3.28	0.6 0.7	2.3	2.5	6.1
8	"	4	2	40.0	6200	248.0	680	9.1	1.02	8.65	3.50	3.36	0.4 0.7	1.9	2.3	5.3
1	256	1	2	0.9	6510	5.9	500	13.0	0.36	6.15	8.14	3.70	0.2 0.4	0.9	1.0	2.5
2	"	1	2	0.9	6400	5.8	500	12.8	0.30	6.60	5.58	3.60	0.5 0.4	1.0	1.1	3.0
7	"	4	2	40.0	6300	252.0	550	11.5	0.92	9.46	2.48	3.20	0.8 0.5	2.5	2.5	6.3
8	"	4	2	40.0	6280	251.0	700	9.0	1.09	8.43	3.37	3.28	0.8 0.5	1.9	2.0	5.2
1	257	1	2	0.9	6550	5.9	500	13.0	0.35	6.25	8.53	3.70	0.2 0.5	0.9	1.0	2.6
2	"	1	2	0.9	6090	5.5	100	61.0	0.26	6.58	5.60	3.58	0.2 0.5	1.0	1.3	3.0
7	"	4	2	40.0	6430	257.0	600	10.7	0.94	9.20	2.60	3.24	0.9 0.7	2.1	2.3	6.0
8	"	4	2	40.0	6250	250.0	650	9.6	1.05	8.50	3.62	3.35	0.7 0.5	1.9	2.1	5.2
1	258	1	2	0.9	6310	5.7	250	25.2	0.30	6.28	7.83	3.60	0.2 0.5	0.9	1.1	2.7
2	"	1	2	0.9	5720	5.2	250	22.9	0.34	6.50	4.79	3.50	0.3 0.6	1.0	1.5	3.0
7	"	4	2	40.0	6000	240.0	680	8.8	0.96	9.15	2.58	3.34	0.9 0.6	2.0	2.3	5.8
8	"	4	2	40.0	6300	252.0	570	11.0	1.00	8.38	3.65	3.48	0.7 0.6	1.6	2.0	4.9
1	259	1	2	0.9	6400	5.8	000	0.0	0.39	6.25	7.90	3.60	0.2 0.4	1.1	1.0	2.7
2	"	1	2	0.9	5700	5.1	250	22.8	0.32	6.55	5.20	3.53	0.3 0.4	1.0	1.3	3.0
7	"	4	2	40.0	5820	233.0	600	9.7	0.95	9.25	2.55	3.24	0.7 0.5	2.2	2.6	6.0
8	"	4	2	40.0	6150	246.0	700	8.8	1.03	8.35	3.47	3.35	0.7 0.5	1.8	2.0	5.0
1	250	2	2	40.0	7330	293.0	200	36.6	1.20	6.20	9.40	3.72	0.2 0.5	0.8	1.0	2.5
2	"	2	2	40.0	7350	294.0	300	24.5	1.18	6.24	9.45	3.84	0.2 0.5	0.8	0.9	2.4
7	"	3	2	0.9	6320	6.1	350	19.5	0.37	6.25	6.64	3.80	0.2 0.5	0.8	1.0	2.5
8	"	3	2	0.9	6550	5.9	550	11.9	0.26	6.50	4.44	3.82	0.3 0.4	1.0	1.0	2.7
1	251	2	2	40.0	7280	291.0	500	14.6	1.16	6.00	10.00	3.60	0.2 0.4	0.8	1.0	2.4
2	"	2	2	40.0	7300	292.0	200	36.5	1.16	6.10	9.44	3.75	0.2 0.3	0.9	1.0	2.4
7	"	3	2	0.9	6800	6.1	200	34.0	0.36	6.30	6.73	3.84	0.2 0.5	0.8	1.0	2.5
8	"	3	2	0.9	6500	5.8	650	10.0	0.28	6.45	4.68	3.87	0.3 0.5	0.8	1.0	2.6
1	252	2	2	40.0	7300	292.0	300	24.3	1.15	6.40	8.25	3.81	0.2 0.5	0.9	1.0	2.6
2	"	2	2	40.0	7320	293.0	350	21.0	1.18	6.45	8.18	3.95	0.2 0.4	0.9	1.0	2.5
7	"	3	2	0.9	6750	6.1	300	22.5	0.46	6.30	6.48	3.81	0.2 0.4	0.9	1.0	2.5
8	"	3	2	0.9	6480	5.8	500	13.0	0.26	6.58	4.32	3.98	0.2 0.5	0.9	1.0	2.6
1	253	2	2	40.0	7500	300.0	400	19.0	1.18	6.25	8.73	3.75	0.2 0.4	0.9	1.0	2.5
2	"	2	2	40.0	7400	296.0	350	21.0	1.14	6.20	8.67	3.81	0.2 0.5	0.8	0.9	2.4
7	"	3	2	0.9	6840	6.2	300	23.0	0.45	6.32	6.90	3.91	0.3 0.4	0.7	1.0	2.4
8	"	3	2	0.9	6580	5.9	400	16.5	0.18	6.60	4.50	4.00	0.3 0.6	0.7	1.0	2.6

First two weeks of each period in trial of zinc deficiency versus zinc normal (continued)

Weeks	Lamb No.	Treat-ment	Sex	Zinc in diet	Weekly mean			F.C.E.	Mean plasma values per week			Mean plasma values per week g/100 ml					
					Food intake	Zinc intake	Growth rate		Zinc	Protein	Alk.phosph.	Albumin	α_1	α_2	β-glob.	γ-glob.	Total globulin
				mg/kg	g	mg	g		µg/ml	g/100 ml	Sigma units						
1	254	2	2	40.0	7300	292.0	400	18.3	1.22	6.20	9.85	3.74	0.2	0.4	0.9	1.0	2.5
2	"	2	2	40.0	7340	294.0	400	18.3	1.20	6.38	9.62	3.85	0.3	0.4	0.8	1.0	2.5
7	"	3	2	0.9	6800	6.1	200	34.0	0.48	6.35	6.50	3.89	0.2	0.5	0.8	1.0	2.5
8	"	3	2	0.9	6600	5.9	500	13.2	0.23	6.58	4.55	3.85	0.3	0.7	0.7	1.0	2.7
1	260	5	2	40.0	6300	252.0	150	42.0	1.18	6.25	8.67	3.78	0.2	0.5	0.8	1.0	2.5
2	"	5	2	40.0	6100	244.0	800	7.6	1.16	6.19	8.53	3.76	0.2	0.4	0.8	1.0	2.4
7	"	6	2	40.0	6820	273.0	700	9.8	1.13	6.25	8.40	3.85	0.2	0.3	0.9	1.0	2.4
8	"	6	2	40.0	6550	262.0	600	11.0	1.14	6.32	8.38	3.91	0.2	0.4	0.8	1.0	2.4
1	261	5	2	40.0	6510	260.0	450	14.5	1.21	6.30	8.53	3.78	0.2	0.4	0.9	1.0	2.5
2	"	5	2	40.0	6400	256.0	750	8.1	1.23	6.38	8.43	3.88	0.2	0.4	0.9	1.0	2.5
7	"	6	2	40.0	6800	272.0	900	7.6	1.12	6.30	8.45	3.91	0.2	0.5	0.7	1.0	2.4
8	"	6	2	40.0	6500	260.0	500	13.0	1.10	6.25	8.29	3.78	0.2	0.4	0.9	1.0	2.5
1	262	5	2	40.0	6550	262.0	080	82.0	1.16	6.20	8.35	3.66	0.2	0.3	1.0	1.0	2.5
2	"	5	2	40.0	6090	244.0	800	7.6	1.17	6.25	8.36	3.68	0.2	0.3	1.0	1.0	2.6
7	"	6	2	40.0	6750	270.0	700	9.7	1.16	6.18	8.67	3.70	0 2	0.5	0.8	1.0	2.5
8	"	6	2	40.0	6480	259.0	600	10.8	1.14	6.18	8.53	3.73	0.2	0.5	0.8	1.0	2.4
1	263	5	2	40.0	6310	252.0	150	42.0	1.15	6.45	9.58	3.88	0.3	0.4	0.9	1.0	2.6
2	"	5	2	40.0	5720	229.0	800	7.2	1.20	6.35	10.10	3.87	0.3	0.5	0.7	1.0	2.5
7	"	6	2	40.0	6840	274.0	800	8.6	1.15	6.35	10.08	3.81	0.2	0.4	0.9	1.0	2.5
8	"	6	2	40.0	6580	263.2	-200	33.0	1.18	6.30	10.10	3.78	0.2	0.4	0.9	1.0	2.5
1	264	5	2	40.0	6400	256.0	100	64.0	1.16	6.20	10.35	3.78	0.2	0.5	0.7	1.0	2.4
2	"	5	2	40.0	5700	228.0	850	6.7	1.17	6.15	10.27	3.75	0.2	0.5	0.7	1.0	2.4
7	"	6	2	40.0	6800	272.0	700	9.7	1.13	6.25	10.20	3.81	0.2	0.5	0.7	1.0	2.4
8	"	6	2	40.0	6600	264.0	-100	66.0	1.12	6.30	10.10	3.84	0.2	0.5	0.8	1.0	2.5
1	724	1	1	0.9	4900	4.4	-500	9.8	0.45	5.90	9.30	3.42	0.4	0.7	0.5	0.7	2.5
2	"	1	1	0.9	4090	3.7	400	10.2	0.30	6.31	8.70	3.28	1.0	0.6	0.7	0.8	3.0
7	"	4	1	40.0	5380	215.0	300	18.0	0.65	8.28	2.40	3.18	1.5	0.6	1.2	1.8	5.1
8	"	4	1	40.0	4580	183.0	500	9.2	0.84	7.38	6.70	3.23	1.2	0.6	0.9	1.4	4.1
1	796	1	1	0.9	6000	5.4	000	60.0	0.40	6.80	9.00	3.80	0.4	0.7	1.1	0.8	3.0
2	"	1	1	0.9	6000	5.4	300	20.0	0.28	7.50	8.80	3.40	1.0	0.3	1.4	1.4	4.1
7	"	4	1	40.0	5500	220.0	900	6.1	0.68	9.27	2.80	3.20	1.6	0.8	1.6	2.0	6.1
8	"	4	1	40.0	6100	244.0	900	6.8	0.88	8.58	6.40	3.25	1.2	0.8	1.5	1.8	5.3
1	781	1	1	0.9	5000	4.5	400	12.5	0.38	6.66	9.50	3.63	0.5	0.8	0.9	0.8	3.0
2	"	1	1	0.9	5700	5.1	200	28.5	0.30	7.20	9.00	3.45	0.5	0.9	1.3	1.1	3.7
7	"	4	1	40.0	5100	204.0	600	8.5	0.69	9.93	2.80	3.10	2.0	1.0	1.7	2.1	6.8
8	"	4	1	40.0	5400	216.0	800	6.8	0.82	9.16	6.50	3.24	1.5	1.0	1.5	1.9	5.9

First two weeks of each period in trial of zinc deficiency versus zinc normal (continued) Appendix 22

Weeks	Lamb No.	Treatment	Sex	Zinc in diet mg/kg	Weekly mean: Food intake g	Weekly mean: Zinc intake mg	Weekly mean: Growth rate g	F.C.E.	Mean plasma values per week: Zinc µg/ml	Mean plasma values per week: Protein g/100 ml	Mean plasma values per week: Alk.phospht. Sigma units	Mean plasma values per week g/100 ml: Albumin	α1	α2	β-glob.	γ-glob.	Total globulin
1	839	1	1	0.9	6000	5.4	-400		0.43	6.96	9.30	4.00	0.5	0.7	0.7	1.0	2.9
2	"	1	1	0.9	6200	5.6	200	31.0	0.31	7.55	8.50	3.80	0.4	0.6	1.3	1.4	3.7
7	"	4	1	40.0	5600	224.0	500	11.2	0.72	9.06	2.60	3.10	1.4	0.7	1.6	2.2	5.9
8	"	4	1	40.0	5200	208.0	700	7.4	0.90	8.38	6.80	3.28	1.2	0.8	1.3	1.7	5.1
1	823	1	1	0.9	6200	5.6	-200		0.41	6.05	8.60	3.44	0.3	0.6	1.0	0.7	2.6
2	"	1	1	0.9	6000	5.4	200	30.0	0.29	7.47	8.40	3.32	0.6	0.7	1.4	1.4	4.1
7	"	4	1	40.0	5300	212.0	700	7.6	0.70	8.40	3.00	2.85	0.9	0.7	1.8	2.1	5.5
8	"	4	1	40.0	5000	200.0	900	5.6	0.88	8.30	6.60	3.05	0.9	0.7	1.6	2.0	5.2
1	750	2	1	40.0	6000	240.0	-700		1.20	6.32	9.20	3.90	0.3	0.4	0.8	0.9	2.4
2	"	2	1	40.0	6900	276.0	500	13.8	1.15	6.12	8.80	3.75	0.2	0.6	0.7	0.9	2.4
7	"	3	1	0.9	6400	5.8	800	8.0	0.43	6.35	5.60	3.75	0.3	0.6	0.7	1.0	2.6
8	"	3	1	0.9	5300	4.8	000	53.0	0.22	7.35	3.40	3.30	0.4	0.9	1.3	1.4	4.0
1	860	2	1	40.0	5900	236.0	300	19.7	1.00	7.05	10.00	4.34	0.2	0.4	0.7	1.4	2.7
2	"	2	1	40.0	5500	220.0	500	11.0	0.94	7.00	9.70	4.45	0.3	0.6	0.7	0.9	2.5
7	"	3	1	0.9	6000	5.4	800	7.5	0.48	6.90	5.80	4.20	0.2	0.4	0.8	1.3	2.7
8	"	3	1	0.9	5100	4.6	300	17.0	0.24	7.64	3.50	4.00	0.3	0.66	0.9	1.8	3.6
1	851	2	1	40.0	6000	240.0	-300		1.14	6.34	7.00	3.86	0.2	0.6	0.7	1.0	2.5
2	"	2	1	40.0	5300	212.0	300	18.0	1.15	6.17	7.00	3.81	0.2	0.6	0.7	0.9	2.3
7	"	3	1	0.9	4700	4.2	1100	4.3	0.41	6.76	5.80	3.76	0.2	0.7	0.9	1.2	3.0
8	"	3	1	0.9	4200	3.8	-200		0.28	7.52	3.30	3.72	0.4	0.8	1.2	1.4	3.8
1	859	2	1	40.0	6300	252.0	000	63.0	1.20	6.20	9.00	3.78	0.2	0.6	0.7	0.9	2.4
2	"	2	1	40.0	5400	216.0	500	11.0	1.15	6.58	9.20	4.02	0.2	0.5	0.7	1.2	2.5
7	"	3	1	0.9	5400	4.9	1100	5.0	0.38	7.20	5.40	4.21	0.4	0.8	0.9	0.9	3.0
8	"	3	1	0.9	5000	4.5	500	10.0	0.20	7.93	3.10	4.19	0.4	0.9	1.1	1.3	3.7
1	708	2	1	40.0	6100	244.0	300	20.3	1.21	6.74	8.50	4.12	0.2	0.6	0.7	1.1	2.6
2	"	2	1	40.0	7300	292.0	-200		1.09	6.70	9.00	4.12	0.3	0.7	0.8	0.8	2.6
7	"	3	1	0.9	5700	5.1	1000	5.7	0.40	6.85	5.80	4.00	0.4	0.6	0.9	1.0	2.8
8	"	3	1	0.9	5400	4.9	400	13.5	0.20	7.05	3.40	3.80	0.5	0.8	1.0	1.0	3.2
1	718	5	1	40.0	4900	196.0	-100		1.20	6.24	9.00	3.80	0.2	0.3	0.9	1.0	2.4
2	"	5	1	40.0	4090	164.0	600	6.8	1.15	6.13	8.50	3.68	0.2	0.4	0.9	1.0	2.4
7	"	6	1	40.0	6400	256.0	1500	4.3	1.16	6.10	8.80	3.65	0.3	0.2	0.9	1.1	2.4
8	"	6	1	40.0	5300	212.0	1050	5.1	1.18	6.14	8.60	3.70	0.2	0.4	0.8	1.0	2.4
1	722	5	1	40.0	6000	240.0	300	20.0	1.08	6.42	9.80	3.80	0.1	0.3	0.8	1.4	2.6
2	"	5	1	40.0	6000	240.0	500	12.0	1.10	6.36	9.50	3.75	0.2	0.3	0.8	1.3	2.6
7	"	6	1	40.0	6000	240.0	650	9.2	1.08	6.48	9.20	3.60	0.3	0.4	0.7	1.5	2.9
8	"	6	1	40.0	5100	204.0	1100	4.6	1.06	6.50	8.80	3.65	0.2	0.5	0.8	1.3	2.8

First two weeks of each period in trial of zinc deficiency versus zinc normal (continued)

Appendix 23

Weeks	Lamb No.	Treatment	Sex	Zinc in diet mg/kg	Weekly mean: Food intake g	Weekly mean: Zinc intake mg	Weekly mean: Growth rate g	F.C.E.	Mean plasma values per week: Zinc µg/ml	Mean plasma values per week: Protein g/100 ml	Mean plasma values per week: Alk.phospht. Sigma units	Mean plasma values per week g/100 ml: Albumin	∝1	∝2	β-glob.	γ-glob.	Total globulin
1	734	5	1	40.0	5000	200.0	400	12.5	1.20	5.72	6.00	3.50	0.2	0.4	0.7	0.9	2.2
2	"	5	1	40.0	5700	228.0	300	19.0	1.16	5.55	5.80	3.60	0.2	0.4	0.6	0.8	2.0
7	"	6	1	40.0	4700	188.0	1650	2.8	1.15	5.17	5.30	3.40	0.3	0.4	0.7	0.7	2.0
8	"	6	1	40.0	4200	168.0	750	8.3	1.16	5.05	5.50	3.35	0.2	0.4	0.7	0.7	2.0
1	788	5	1	40.0	6000	240.0	500	12.0	1.10	6.30	8.90	3.60	0.2	0.5	0.9	1.1	2.7
2	"	5	1	40.0	6200	248.0	500	12.4	1.09	6.25	9.20	3.55	0.2	0.5	0.8	1.2	2.7
7	"	6	1	40.0	5400	216.0	1200	4.5	1.05	6.35	8.50	3.95	0.2	0.3	0.9	1.0	2.4
8	"	6	1	40.0	5000	200.0	880	5.7	1.04	6.20	8.40	3.85	0.3	0.3	0.8	1.0	2.4
1	809	5	1	40.0	6200	248.0	800	7.8	1.00	6.28	10.00	3.80	0.2	0.5	0.9	0.9	2.5
2	"	5	1	40.0	6000	240.0	400	15.0	1.04	6.25	9.80	3.90	0.2	0.5	0.7	1.0	2.4
7	"	6	1	40.0	5700	228.0	600	9.5	1.03	6.26	9.60	3.90	0.3	0.5	0.7	0.9	2.4
8	"	6	1	40.0	5400	216.0	700	7.7	1.00	6.18	9.80	3.80	0.3	0.4	0.7	1.0	2.4